Hjertefunksjon og kardiovaskulære risikomarkører hos barn og unge voksne som har overlevd alvorlig sykdom

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Avhandling for graden philosophiae doctor (ph.d.)
Universitetet i Bergen
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1. Scientific environment

The research work presented in this thesis was conducted as part of the PhD programme at the Department of Clinical Science, Faculty of Medicine, University of Bergen, Norway. The research was part of the national study Project Extreme Prematurity (PEP), affiliated to the Westpaed Research Group at Haukeland University Hospital in Bergen, and the international study Physical Activity and Fitness in Childhood Cancer Survivors (PACCS), affiliated to Oslo University Hospital, Norway, The Norwegian School of Sport Sciences, and Haukeland University Hospital in Bergen, Norway.

PhD Elisabeth Leirgul, cardiologist at Department of Heart Disease at Haukeland University Hospital, Bergen, Norway, was the main supervisor for this PhD project, and Professor Gottfried Greve, vice-rector at University of Bergen and paediatrician at Haukeland University Hospital in Bergen, Norway, was co-supervisor.

Statistical analyses were performed in collaboration with biostatistician Karl Ove Hufthammer and Fatemeh Zamanzad Ghavidel at Centre for Clinical Research, Haukeland University Hospital in Bergen, Norway.

The PhD project was funded by the Norwegian Association for Children with Heart Disease, Bergen Heart Foundation at University of Bergen, Norway, the Childhood Cancer Society in Norway, and the Department for Heart Disease at Haukeland University Hospital in Bergen, Norway.

2. Acknowledgements

First and foremost, I would like to thank my main supervisor, Elisabeth Leirgul, for being an inspiring, excellent, and patient mentor during these last years. She has enthusiastically shared of her extensive knowledge in many fields, led me securely through the different stages of my PhD, and given me extremely precise and constructive feedback during my work. She has taught me how to find answers, but more importantly, also to look for questions, and to value evidence over ever so great opinions. Additionally, receiving text messages from me at all times of the day did not seem to push her off the floor (although maybe wake her up early Sunday mornings).

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I will also thank all the co-workers in the PEP and PACCS projects, including the study-nurses Merete Benestad and Sunni Helland, and my co-authors. A special thanks to my co-author Tom Roar Omdal for supporting me with inter-rater analyses and for always being helpful and a true “yes person”. Additionally, a particular thanks to Mette Engan for countless short (seconds) phone calls regarding research-related questions that according to my impatient natur could not wait to be answered. Thanks also to the two statisticians, Fatemeh Ghavidel and Karl-Ove Hufthammer, helping me through the winding paths of statistics, they have indeed taught me much!

I will express my gratitude to the University of Bergen, which gave me the opportunity to enter the world of science and climb the academic ladder, and to the Research Council of Norway which covered the expenses for running the PACCS study, and the Western Norway Health Authority which covered the expenses for

running the long-term follow-up of the PEP study. In addition, I will express my gratitude to the Bergen Heart Foundation at University of Bergen, the Norwegian Association for Children with Heart Disease, the Childhood Cancer Society in Norway, and the Department of Heart Disease at Haukeland University Hospital in Bergen. Their generous financial support made this project achievable.

In my daily work as a cardiologist at Haukeland University Hospital, I meet tiny preterm born struggling in the incubators, and brave children diagnosed with cancer. In my eyes they are all heroes. After having had the opportunity to deepen my knowledge in the field of their cardiovascular late effects, it will be important for me to keep working towards securing a better long-term follow-up for these young individuals who managed to weather the storm at the beginning of their life. My thankful thoughts go to the study participants, without their willingness and effort, this project would not have been possible!

Anja and Annabel, my good-as-gold office mates, thank you both for bringing a humorous breath of fresh air into our (exceptionally tidy) office, and to Elisabeth S, my kind ex-office mate, thank you for always sharing and cheering.

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The most important and heartfelt thanks to my lovely children, Oda and Oskar, who brighten my day, and to my dear husband Thomas, my favorite person and greatest support, who gave me the ultimate jackpot by adding three more children to the breakfast table. Together is our favorite place to be!

(I would also thank my running shoes and the stairs of Ulriken rescuing me from complete state of catabolism facing the computer for hours and hours during the completion of this thesis).
-Bergen, May 2023-

3. Abstract

Background

The underlying processes of cardiovascular disease begin early in life and depend on the number and intensity of risk factors. Some populations are prone to increased cardiovascular risk already at young age due to severe disease and intensive treatment during development and growth, such as survivors of preterm birth and childhood cancer. Clinical studies of their cardiovascular function are of importance to increase knowledge of their cardiovascular risk.

Methods

The "Project Extreme Prematurity" (PEP) has included three Norwegian cohorts of young adults born very preterm at gestational age <29 weeks or with extremely low birthweight <1000 grams (PB/ELBW), and sex- and age matched term-born controls. Endothelial function, assessed by ultrasound measured flow-mediated dilatation (FMD) of the brachial artery, was investigated in 50 PB/ELBW with mean age 28 years (± 6) and 49 controls, and myocardial function, assessed by echocardiography, was investigated in 63 PB/ELBW with mean age 27 years (± 6) and 64 controls of this study population.

The "Physical Activity and fitness in Childhood Cancer Survivors" (PACCS) study has included paediatric childhood cancer survivors (CCSs) previously treated for cancer in Norway, and sex- and age matched controls. Echocardiographic measured myocardial function was investigated in 128 CCSs with mean age 14 years (± 3) and 23 controls of this study population. Additionally, the correlation between myocardial function and peak oxygen consumption (VO_2) achieved on treadmill was analysed in the CCSs.

Results

The PB/ELBW had reduced endothelial function and systolic myocardial function compared to controls. FMD was 5.4% (95% confidence interval (CI) 4.2, 6.6) for PB/ELBW versus 7.5% (95% CI 6.2, 8.9) for controls, p= 0.02. Left ventricular global longitudinal strain (LV-GLS) was -19.4% (95% CI -20.0, -18.9) for PB/ELBW versus -20.6% (95% CI -21.1, -20.1) for controls, p= 0.003. Measures of diastolic

function, including left atrial (LA) reservoir strain, and measures of myocardial work were similar in PB/ELBW and controls.

The CCSs had reduced LV-GLS compared to controls (-19.7% (95% CI -20.1, -19.3) versus -21.3% (95% CI -22.2, -20.3), respectively, p= 0.004). This also applied for the CCS subgroup treated with low doses of anthracyclines (<100 mg/m^2) which had lower LV longitudinal strain z-score compared to the controls (-0.1 (95% CI -0.4, 0.5) versus 0.9 (95% CI 0.2, 1.5), respectively, p= 0.02). Among the CCSs, lower left ventricular myocardial function was associated with lower peak VO_2 (PCC= -0.3 for LV-GLS), and higher doses of anthracyclines and increasing time after treatment were associated with lower myocardial function (PCC= 0.5 and 0.3, respectively, for LV-GLS).

Conclusions and future perspectives

The PB/ELB had lower endothelial function and myocardial function measured by LV-GLS compared to controls. These findings could indicate an elevated risk in preterm born young adults of developing atherosclerotic cardiovascular disease and heart failure.

The paediatric CCSs, including the subgroup treated with low doses of anthracyclines, had reduced LV systolic myocardial function compared to controls, and lower LV function was associated with poorer peak VO_2. Increasing time after treatment and higher treatment doses of anthracyclines were associated with lower myocardial function. These findings could indicate an increased risk of developing heart failure and poorer prognosis with higher treatment doses of anthracyclines, although no safe dose of anthracyclines seems to exist.

Based on these findings, follow-up programs for preterm born adults and survivors of childhood cancer, with special attention to reduce modifiable cardiovascular risk factors, should be recommended, and regularly monitoring of myocardial function might be indicated for all CCSs, regardless of anthracycline treatment doses.

4. Sammendrag på norsk

Bakgrunn

Prosessene som ligger bak utvikling av hjerte-kar sykdom starter tidlig i livet og er avhengig av antall risikofaktorer og debuttidspunkt av disse. Noen grupper er mer utsatt for hjerte-kar sykdom allerede i ung alder på grunn av tidligere medisinsk behandling og sykdom, som for eksempel prematurt fødte og barnekreftoverlevere. For å få mer kunnskap om deres hjerte- og kar helse er det nødvendig med flere kliniske studier.

Metoder

Forskningsprosjektet «Prosjekt Ekstrem Prematuritet» (PEP) har inkludert tre norske kohorter av unge voksne som ble født før svangerskapsuke 29 eller med ekstremt lav fødselsvekt (<1000 gram) (PB/ELBW), samt kjønns- og alderslike terminfødte kontroller. Endotelfunksjon, vurdert ved ultralydmålt stressmediert dilatasjon (FMD) av arteria brachialis, ble målt hos 50 PB/ELBW med gjennomsnittsalder 28 år (± 6) og 49 kontroller, og myokardfunksjon, vurdert ved ekkokardiografi, ble analysert hos 63 PB/ELBW med gjennomsnittsalder 27 år (± 6) og 64 kontroller fra denne studiepopulasjonen.

Studien "Fysisk aktivitet og fysisk form hos barnekreftoverlevere" (PACCS) har inkludert unge barnekreftoverlevere som har vært behandlet for kreft i Norge, samt kjønns- og alderslike kontrollpersoner. Myokardfunksjon, vurdert ved ekkokardiografi, ble analysert hos 128 barnekreftoverlevere med gjennomsnittsalder 14 år (± 3) og 23 kontroller fra denne studiepopulasjonen. I tillegg ble korrelasjonen mellom barnekreftoverlevernes myokardfunksjon og maksimale oksygenopptak (VO_2) oppnådd ved tredemølletest undersøkt.

Resultat

FMD og systolisk myokardfunksjon var lavere hos PB/ELBW sammenlignet med kontrollene. FMD var 5.4% (95% konfidensintervall (CI) 4.2, 6.6) hos PB/ELBW versus 7.5% (95% CI 6.2, 8.9) hos kontrollene, p-verdi 0.02. Venstre ventrikkel global longitudinell strain (LV-GLS) var -19.4% (95 % CI -20.0, -18.9) hos PB/ELBW versus -20.6% (95 % CI -21.1, -20.1) hos kontrollene, p-verdi 0.003.

Diastolisk hjertefunksjon, inkludert analyser av venstre atrie strain, samt ikke-invasivt målte estimater for myokardarbeid var likt hos PB/ELBW og kontrollene.

Venstre ventrikkels systoliske funksjon var redusert hos barnekreftoverleverne sammenlignet med kontrollene. LV-GLS var -19.7% (95% CI -2.1, -19.3) hos barnekreftoverleverne versus -21.3% (95% CI -22.2, -20.3) hos kontrollene, p-verdi 0.004. Barnekreftoverlevere behandlet med lav dose antracykliner (<100 mg/m 2) hadde også lavere z-skår for venstre ventrikkel strain sammenlignet med kontrollgruppen (henholdsvis -0.1 (95% CI -0.4, 0.5) og 0,9 (95% CI 0.2, 1.5), p-verdi= 0.02). Hos barnekreftoverleverne var redusert venstre ventrikkelfunksjon assosiert med lavere maksimalt VO_2 (PCC -0.3 for LV-GLS). Høyere antracyklindoser, og lengre tidsintervall siden siste kreftbehandling var assosiert med avtagende myokardfunksjon (PCC henholdsvis 0.5 og 0.3 for LV-GLS).

Konklusjoner og fremtidsperspektiver

PB/ELBW hadde lavere endotelfunksjon og venstre ventrikkel funksjon sammenlignet med terminfødte kontroller. Disse funnene kan indikere at unge voksne født veldig prematurt eller med ekstremt lav fødselsvekt har økt risiko for å utvikle aterosklerotisk hjerte-karsykdom og hjertesvikt.

Vi fant redusert venstre ventrikkel systolisk funksjon hos unge barnekreftoverlevere sammenlignet med friske kontroller, og lavere venstre ventrikkelfunksjon var assosiert med lavere maksimalt VO_2. Disse funnene indikerer økt risiko for utvikling av hjertesvikt. Lengre tidsintervall siden siste kreftbehandling og høyere behandlingsdoser med antracykliner var assosiert med mer redusert hjertefunksjon, men hjertefunksjonen hos de behandlet med lave antracyklindoser var også redusert sammenlignet med kontrollgruppen.

På bakgrunn av tidligere kunnskap og våre funn anbefaler vi oppfølging av prematurt fødte voksne og unge barnekreftoverlevere, spesielt med tanke på å redusere forekomst av modifiserbare kardiovaskulære risikofaktorer. Regelmessig kontroll av myokardfunksjon hos alle barnekreftoverlever allerede fra ung alder, også hos de behandlet med lave anthracyklindoser, synes også indisert.

5. List of publications

Paper I: Vascular Endothelial Function Assessed by Flow-Mediated Vasodilatation in Young Adults Born Very Preterm or With Extremely Low Birthweight:
A Regional Cohort Study
Britt Engan, Mette Engan, Gottfried Greve, Maria Vollsæter, Karl Ove Hufthammer, Elisabeth Leirgul
Frontiers in Pediatrics, 2021 Sep 24; 9:734082. doi:10.3389/fped.2021.734082 [1]

Paper II: Myocardial function including estimates of myocardial work in young adults born very preterm or with extremely low birthweight
- a cohort study
Britt Engan, Tom Roar Omdal, Gottfried Greve, Maria Vollsæter, Elisabeth Leirgul
BMC Cardiovascular Disorders 2023 April 29; 23:222. doi:10.1186/s12872-023-03253-4 [2]

Paper III: Systolic myocardial function measured by echocardiographic speckle-tracking and peak oxygen consumption in pediatric childhood cancer survivors
-a PACCS study
Britt Engan, Simone Diab, Henrik Brun, Truls Raastad, Ingrid Torsvik, Tom Roar Omdal, Fatemeh Zamanzad Ghavidel, Gottfried Greve, Ellen Ruud, Elisabeth Edvardsen, Elisabeth Leirgul
Frontiers in Cardiovascular Medicine-Cardiovascular Imaging 2023 July 5; 10:1221787. doi:10.3389/fcvm.2023.1221787

6. Abbreviations

ANCOVA- analyses of covariance

BSA- body surface area

CCSs- childhood cancer survivors

CI- confidence interval

EF- ejection fraction

FMD- flow mediated dilatation

GA- gestational age

GCW- global constructive work

GLS- global longitudinal strain

GWE- global work efficiency

GWI- global work index

GWW- global wasted work

LA- left atrium/atrial

LV- left ventricle/ventricular

MW- myocardial work

NID- nitroglycerine-induced dilatation

PACCS- Physical Activity and fitness in Childhood Cancer Survivors

PB/ELBW- very preterm born or with extremely low birthweight

PEP- Project Extreme Prematurity

PCC- Pearson correlation coefficient

PSI- post systolic index

PSS- post systolic shortening

RV-LS- right ventricular longitudinal strain

SD- standard deviation

7. Introduction

7.1 General introduction

Cardiovascular disease is the leading cause of death globally [3] and generates large health expenses. Although cardiovascular disease mainly affects adults, the underlying processes of cardiovascular disease begin early in life and depend on the onset time, number, and intensity of risk factors [4]. Some populations are prone to increased cardiovascular risk already at young age due to severe disease and intensive treatment during development and growth, such as survivors of preterm birth and childhood cancer. Preterm birth and low birthweight have been associated with increased prevalence of cardiovascular disease and death in young adulthood [5, 6], and cardiovascular disease is the leading non-cancer contributor to early morbidity and mortality in childhood cancer survivors (CCSs) [7]. Due to improvements in neonatal intensive care and cancer treatment over the last decades, survival rates for neonates born extremely preterm and children affected by cancer have increased, and large cohorts of these individuals are now growing up. Neonates born extremely preterm now represent approximately 0.5% of all children growing up in Norway [8], and about 6000 have survived childhood cancer in Norway since the early 1980s [9]. Clinical studies of cardiovascular function in preterm born and CCSs are of importance to increase knowledge of their cardiovascular health. This would facilitate adequate follow-up and eventually reduce mortality and the health-economic burden associated with cardiovascular disease.

7.1.1 Preterm birth and low birthweight and the cardiovascular system

7.1.1.1 Definitions and terminology

The normal duration of a pregnancy is 40 weeks, counting from the first day of the last menstrual period. A reliable method to estimate the gestational age (GA) is by ultrasound measurements of foetal biometry before 22 weeks of gestation [10]. Preterm birth is defined as birth before 37 weeks of gestation [11]. Further classification of preterm birth by GA and categorisation of the neonates by birthweight are described in Table 1. The birthweight can also be classified as small, appropriate, or large for GA as percentiles for birthweight by GA have been presented [12, 13].

Table 1 World Health Organization classification of prematurity and birthweight [3]

Extremely preterm born	Gestational age <28 weeks
Very preterm born	Gestational age 28-32 weeks
Moderate preterm born	Gestational age 32-34 weeks
Late preterm born	Gestational age 34-37 weeks
Term born	Gestational age 37-42 weeks
Post-term born	Gestational age ≥42 weeks
Extremely low birthweight	<1000 g
Very low birthweight	1000 g-1500 g
Low birthweight	1500 g-2500 g
Normal birthweight	≥2500 g-4500 g
High birthweight	≥4500 g

7.1.1.2 Risk factors for preterm birth and low birthweight

Approximately two-thirds of preterm births are due to spontaneous labour including preterm rupture of the chorio-amniotic membranes, and one-third are due to medical induction of labour or caesarean delivery due to foetal or maternal indications [14]. Common foetal indications for medical induction of preterm birth include intrauterine growth restriction, insufficient umbilical blood flow, intrauterine infection, or placenta abruption, and common maternal indications are pre-eclampsia, eclampsia,

or HELLP syndrome (haemolysis, elevated liver enzymes, and low platelets) [15]. The aetiology and pathological processes behind spontaneous preterm birth are still object of study. However, multiple mechanisms, including infection and inflammation, uteroplacental ischaemia or haemorrhage, uterine overdistention, and hormonal imbalance (for instance of progesterone and cortisol) have been assumed to trigger preterm labour [14, 15]. Several risk factors are associated with spontaneous preterm birth and low birthweight such as maternal age (<20 and >40 years of age) [16], nulliparity, multiple gestations, former preterm delivery, in vitro fertilization, black skin colour, low educational and socio-economic level, stress, malnutrition, smoking, and use of illicit drugs. Additionally, some maternal cardiovascular risk factors are known to increase the risk of spontaneous preterm birth such as hypertension, diabetes mellitus, pre-eclampsia, and obesity [11].

7.1.1.3 Epidemiology

The last decades, the annual incidence of preterm birth in Norway has been approximately 5-6.5%, and 0.5% have been born extremely preterm and/or with extremely low birthweight [8], which represent approximately 250-300 neonates annually.

7.1.1.4 Developments in neonatal medicine

In the 1970s, most neonates born extremely preterm in Norway did not survive. However, due to establishment of neonatal intensive care units and medical development over the last decades, the survival rates for these neonates today exceed 80% in the Nordic countries [5, 17, 18]. Introduction of and increased use of antenatal corticosteroids and assisted ventilation since the late 1970s, and surfactant since the early 1990s, have been of great importance. Initially, assisted ventilation was mainly in the form of intermittent or continuous positive pressure ventilation via an endotracheal tube. However, since the early 1990s, the use of nasal continuous airway pressure and high frequency oscillatory ventilation have increased [19].

7.1.1.5 Impact of preterm birth on the cardiovascular system

Increasing evidence indicates that children born preterm or with low birthweight carry a risk of poor long-term health outcomes. This applies even to individuals born only moderately to late preterm [5, 6]. Preterm birth and low birthweight are associated with increased prevalence of cardiovascular risk factors [20] such as hypertension, diabetes mellitus and metabolic disease, and a higher risk of cardiovascular disease, all-cause mortality, and young adult death [5, 6, 21-25]. Additionally, large register-based studies have reported increased risk of heart failure already in the adolescent years and in young adulthood [23, 26]. Birthweight and GA are reported to be inversely associated with increased risk of cardiovascular disease [5, 6]. Even though the increased prevalence of cardiovascular risk factors and common genetic factors in the mother and offspring [27] might explain the increased risk of cardiovascular disease, it has been speculated if preterm birth constitutes an independent risk factor for adult cardiovascular disease [28], and the World Health Organization [29] has classified low birthweight as a cardiovascular risk factor.

The underlying mechanisms behind the increased cardiovascular disease rate in preterm born are not fully understood. Various epidemiological studies have suggested that environmental factors early in life influence on future morbidity and mortality [30]. In 1973, the Norwegian physician Forsdahl postulated that poverty and malnutrition in childhood and the adolescent years could increase the risk of cardiovascular disease [31]. In the 1980s, the British physician Barker suggested that the programming of cardiovascular risk starts already in the pre-natal period. He proposed that inadequate nutrition or other causes of impaired in-utero growth, with rapid “catch-up” growth in the post-natal period, influence on body composition and metabolism, and the future risk of metabolic syndrome and cardiovascular disease [28, 30, 32]. However, common genetic factors in the mother and offspring might constitute a greater impact on the future risk of cardiovascular disease than the in-utero environment [27]. Hereditary cardiovascular risk factors such as hypertension, dyslipidaemias, and diabetes mellitus are associated with increased risk of preeclampsia and preterm birth and low birthweight [11, 33], as are pregnancies

complicated by maternal ischaemic heart disease [34].

The post-natal period seems to influence on the increased cardiovascular risk. The preterm birth disrupts intrauterine foetal cardiovascular maturation and leads to early transition from lower-resistance cardio-placental circulation to higher-resistance cardio-pulmonary circulation. The neonates are prone to infections, and treatment with supplemental oxygen and ventilatory support is required due to immature lungs and respiratory distress [30, 35]. Through several mechanisms, this leads to inflammation and increased oxidative stress. In addition to this, an imbalance in the renin-angiotensin-aldosterone system, an immature immune system, and gut microbial dysbiosis, have been suggested to underlie the increased risk of developing cardiovascular diseases [30].

7.1.1.6 Current follow-up routines

Because high survival rates of extreme preterm born have been achieved only recently, the oldest survivors are still young adults. However, large cohorts of extremely preterm born are now entering adult life with cardiovascular health challenges. For the time being, no routine cardiovascular follow-up or preventive strategies are established beyond the usual recommendations for the general population.

7.1.2 Childhood cancer and the cardiovascular system

7.1.2.1 Epidemiology

In the late 1970s and early 1980s, the survival rate of childhood cancer in high income countries was approximately 50%. There have been great improvements in treatment over the last decades, and currently >80% of children diagnosed with cancer are cured [36]. B-cell acute lymphoblastic leukaemia and Hodgkin lymphoma are the most common cancer forms in children and adolescents, respectively, both with a survival rate >90% [37, 38]. In Norway, about 6000 children have survived cancer since the early 1980s [9], and the prevalence of their various cancer diagnoses in year 2021 is described in Table 2.

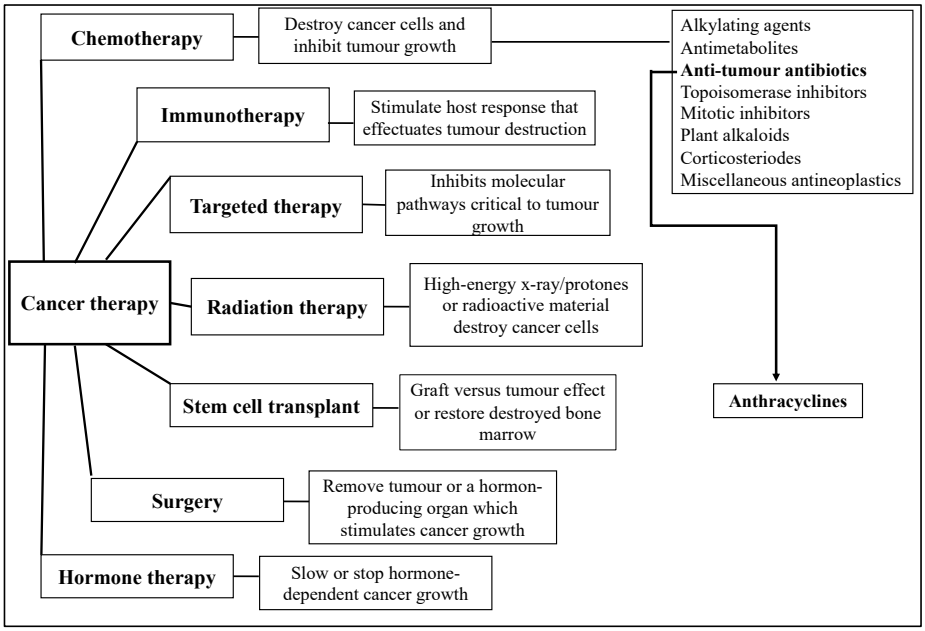
Table 2 Cancer diagnoses in Norwegian childhood cancer survivors year 2021 [9]

Leukaemia	37%
Lymphoma	25%
Cerebrospinal (CNS) tumours	12%
Solid tumours outside CNS	26%

7.1.2.2 Developments in cancer treatment

Cancer treatment has historically consisted of chemotherapy, irradiation, and surgery. Major improvements in diagnostics and treatment of childhood cancer since the 1960s and1970s are largely due to the organization of international multidisciplinary study groups and clinical trials. This has led to introduction of tailored protocol-based combination therapies for different cancer subtypes [36]. Additionally, advances in diagnostics including genetic-based characterization and risk stratification of the cancer, therapy intensification, increasing availability of targeted therapies and immunotherapeutic agents, newer approaches for delivery of irradiation, and advancements in supportive care have increased survival rates [36, 39]. A brief overview of the different cancer therapies and a short description of their antineoplastic effects are described in Figure 1.

Figure 1 Brief summary of different cancer therapies



7.1.2.3 Treatment-related cardiovascular adverse effects

Chemotherapeutic medication and radiation therapy are associated with dose-dependent cardiovascular toxicity [40-43] and increased risk of heart failure [44], particularly when cardiovascular risk factors such as hypertension, diabetes, and dyslipidaemia are present. In addition, concurrent treatment with chemotherapy and irradiation has potentiating cardiotoxic effects [45-47]. Also, the disease process itself might be an independent risk factor for cardiovascular adverse effects, due to chronic inflammation and oxidative stress present in cancer diseases [48].

Anthracyclines are the class of chemotherapeutic drugs with most prominent association with cardiotoxicity [7], and are used for a wide spectrum of childhood cancers [49]. The anthracyclines are known to cause acute and late cardiomyopathy, often irreversible and progressive [50-52], and are also suggested to cause stress-induced cardiomyopathy (takotsubo) [53].

The underlying mechanisms of anthracycline-induced cardiotoxicity are complex, however, involves formation of free radicals during metabolism of the anthracycline due to impairment of the mitochondrial function [53, 54]. Eventually, the increased oxidative stress leads to death or dysfunction of the cardiomyocytes [53]. In recent years, genetic variants associated with substantially increased risk of heart failure in CCS treated with anthracyclines have been identified [44, 55].

Other classes of antineoplastic drugs have mainly been linked to hypertension, thromboembolism, cardiac ischaemia, stroke, myocarditis, but also heart failure [56-59].

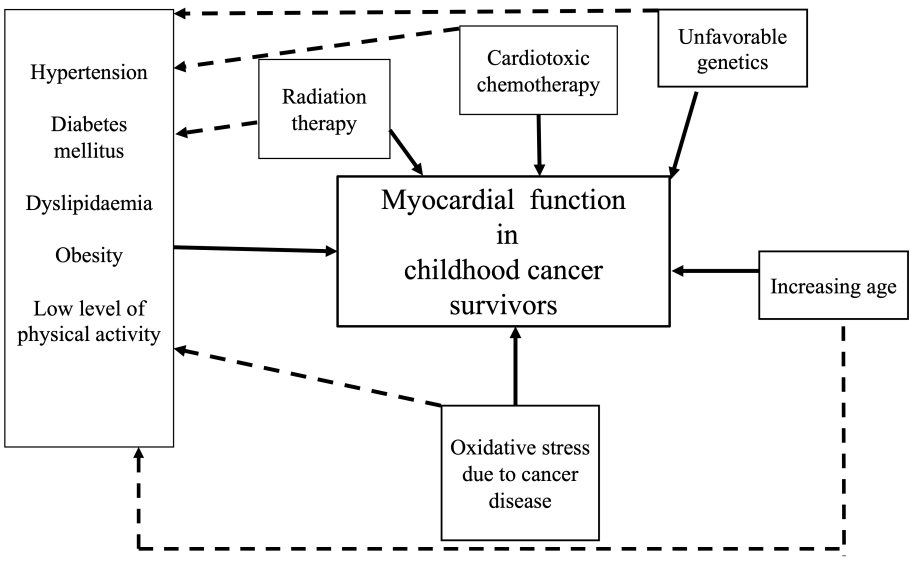
Radiation therapy involving the chest may be responsible for acute and chronic pericardial disease, premature coronary artery disease, cardiomyopathy including restrictive cardiomyopathy, valvular disease, and conduction abnormalities [59]. There are several complex mechanisms behind the cardiotoxic effect of irradiation. Increased oxidative stress due to mitochondrial dysfunction and production of reactive oxygen species, and direct damage of deoxyribonucleic acid (DNA) with activation of apoptosis, result in death of the cardiomyocytes [60]. In addition, the increased oxidative stress results in endothelial dysfunction, epicardial- and microvascular injury, inflammation, and increased probability of thrombosis [60].

7.1.2.4 Cardiovascular health in CCSs

Cardiovascular disease is a leading non-cancer contributor to early morbidity and mortality in CCSs [7]. CCSs have a poorer cardiovascular risk profile than the general population; they are more likely overweight, have more often hypertension and dyslipidaemia, and are less active [61]. Exercise intolerance and decreased maximal oxygen consumption (VO_2) are prevalent among adult CCSs [62, 63], and are reported reduced also in a few small studies of paediatric CCS populations [64-66]. Physical activity and exercise have shown to have a positive preventive effect on cardiovascular disease [67-69], and to possibly prevent development of cancer treatment-related cardiomyopathy in paediatric and adolescent cancer survivors [70].

The maturing cardiovascular system is particularly vulnerable to adverse effects of cancer treatment [71], and the increased prevalence of cardiovascular risk factors already at young age increases the CCSs´ lifetime risk of developing heart failure [44]. Several factors known to influence on the myocardial function of CCSs are described in Figure 2. The cumulative incidence of heart failure 40 years after a cancer diagnosis is reported to be 3% for CCSs treated with irradiation involving the heart, 10% for those treated with cardiotoxic chemotherapy, and approximately 30% for those treated with both cardiotoxic chemotherapy and irradiation involving the heart [72].

Figure 2 Factors influencing myocardial function in childhood cancer survivors [44]



Factors with negative impact on cardiovascular risk factors (dotted lines) and myocardial function (continuous lines) in childhood cancer survivors.

7.1.2.5 Current follow-up routines

The follow-up recommendations of CCSs are based on cardiovascular risk stratification considering risk factors present prior to the cancer diagnosis, cardiotoxic treatment doses (Table 3), and treatment-related cardiovascular adverse effects during

the treatment. The incidence of heart failure is reported to be <5% with anthracycline doses <250 mg/m^2, approximately 10% with doses between 250 and 600 mg/m^2, >30% with doses >600 mg/m^2 [73].

The European Society of Cardiology guidelines on cardio-oncology [74] and The Children's Oncology Group Long-Term Follow-Up Guidelines for Survivors of Childhood, Adolescent, and Young Adult Cancers [75] recommend

- education of all adolescent and adult CCSs and their health care providers to ensure attention to the increased cardiovascular risk
- annual screening for modifiable cardiovascular risk factors in all CCSs
- to consider echocardiographic examination every 2 years in high risk CCSs (Table 3)
- to consider echocardiographic examination every 5 years in moderate risk CCSs (Table 3)

Additionally, cardiovascular assessment is recommended prior to pregnancy and in the first trimester for all female CCSs.

Table 3 Risk categories for development of cardiomyopathy in childhood cancer survivors based on cardiotoxic treatment doses [73, 74, 76]

	Single therapy		Combination therapy	
Risk (incidence)	Radiation therapy to the chest (Gray)	Total cumulative anthracycline dose (mg/m^2)	Radiation therapy to the chest (Gray)	Total cumulative anthracycline dose (mg/m^2)
Very high risk (>20%)	>25	≥400	>15	≥100
High risk (>5%)	<15-25	250-399	5-15	≥100
Moderate risk (2-5%)	5-15	100-249	<5	≥100
Low risk (<2%)	<5	<100	-	-

7.2 Introduction of methods

7.2.1 Endothelial function assessed by flow-mediated dilatation

Blood vessels comprise three layers. The innermost layer is the intima which consists of a single layer of endothelial cells lining the lumen of the vessels (the endothelium) and subendothelial connective tissue. The middle layer, the media, is composed of smooth muscle cells, and the outermost layer, the adventitia, contains connective tissue [77, 78]. A healthy endothelium is important for vascular tone as an appropriate balance of endothelial-dependent relaxing factors (nitric oxide and prostacyclin) and contractile factors (prostaniodes, endothelin-1, and uridine adenosine tetraphosphate) regulates arterial smooth muscle vasodilatation and vasoconstriction [79-82]. Endothelial dysfunction occurs if the endothelium's ability to produce and release nitric oxide is reduced and consequently decreases the relaxing stimuli to the smooth muscle cells[82]. Vascular endothelial dysfunction has appeared to be an early sign of the development of atherosclerosis and a predictor of cardiovascular disease [79, 82-85].

Factors reported to adversely affect the endothelium include common cardiovascular risk factors such as tobacco use, hypertension, obesity, hyperlipidaemia, insulin resistance, and physical inactivity [86]. Both sexes show an age-dependent decline in endothelial function [79], and a steeper decline is seen in postmenopausal women [87]. The underlying mechanisms of why these factors lead to impairment of the endothelium are complex, and still object of study, but they are related to increased production of reactive oxygen species with subsequently increased oxidative stress in the vessel walls [88, 89]. This may induce autophagy of the endothelial cells [88] and reduce bioavailability of nitric oxide due to inactivation of nitric oxide by the reactive oxygen species [89].

In addition, endothelial dysfunction has been reported in patients with a family history of early cardiovascular disease, but without other risk factors [86]. Certain gene expression patterns have been associated with altered coronary artery endothelial function [90], suggesting that genetic regulators influence on endothelial function [91].

Assessment of endothelial function by flow-mediated dilatation (FMD) of the brachial artery uses increased hemodynamic shear stress during reactive hyperaemia

to provoke the release of nitric oxide with subsequent vasodilatation that can be imaged and quantitated by ultrasound [92, 93]. FMD assesses endothelial-dependent vasodilatation and is well established as a method to evaluate macrovascular endothelial function. FMD is reported to correlate with invasive testing of coronary endothelial function, as well as with the severity and extent of coronary atherosclerosis [94]. Nitroglycerine-induced dilatation (NID) of the brachial artery represents endothelial independent vasodilatation. The presence of established cardiovascular disease or multiple cardiovascular risk factors have previously been reported to impair NID of the brachial artery [95], and thus, NID might reflect vascular smooth muscle cell dysfunction and vascular structure alternations due to established atherosclerosis [96, 97]. The interrelationship between endothelial-dependent FMD and endothelial-independent NID, and the relation to cardiovascular risk factors and disease are debated and not fully understood [95, 98-100].

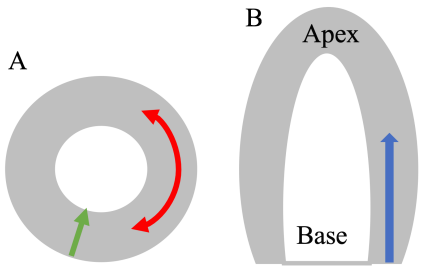
Several factors transiently affect flow-mediated vascular reactivity including temperature, level of sex hormones, digestion of high fat foods, and sympathetic stimuli like vasoactive drugs, nicotine, caffeine, and exercise [93]. These confounding factors must be considered during the assessments of endothelial function by FMD.

7.2.2 Speckle-tracking echocardiography

7.2.2.1 Left and right ventricular longitudinal strain

Cardiac deformation of the left ventricle (LV) during contraction consists of a complex longitudinal, circumferential, and radial motion, often described as a "twisting" motion [101] (Figure 3). The right ventricle (RV) contracts by longitudinal shortening with traction of the tricuspid annulus towards the apex, inward radial movement of the free wall ("bellow effect"), and stretching of the free wall over the septum (causing shortening in the anteroposterior direction) due to bulging of the interventricular septum into the RV during LV contraction [102, 103].

Figure 3 Left ventricular deformation during systole



A: Left ventricle short axis; green line represents radial myocardial fiber motion and red line represents circumferential myocardial fiber motion during systole
B: Left ventricle long axis; blue line represents longitudinal myocardial fiber motion during systole

Speckle-tracking echocardiography allows calculation of myocardial deformation by analysing motions of speckles (spots generated by the interaction between the ultrasound beam and myocardial fibres) during the cardiac cycle. LV and RV longitudinal strain represent myocardial deformation directed from the base to the apex of the ventricles and are described as negative percentages, as they describe myocardial shortening of segments relative to original length [101]. Compared to more conventional echocardiographic parameters of left and right ventricular myocardial function, measures of speckle-tracking derived strain have shown to be more sensitive to changes in regional and global ventricular myocardial function, and to provide additional prognostic value [104-106].

7.2.2.2 Post systolic shortening

Echocardiographic speckle-tracking allows measurements of post systolic shortening (PSS) which represents delayed myocardial deformation after end-systole. PSS is calculated as post systolic index (PSI); the percentage deformation of total deformation that occurs after end-systole. In myocardial disease, PSS is believed to be caused by impaired inherent segment contractility, delayed ventricular conduction, and passive recoil caused by tension from surrounding healthy myocardial segments

[107, 108]. PSS has mostly been described in myocardial segments with contractile dysfunction due to ischaemia, but has also been observed in non-ischaemic heart diseases such as hypertrophic and dilated cardiomyopathy, takotsubo cardiomyopathy, aortic stenosis, and hypertension [109-111]. The extent of PSS is related to the degree of myocardial impairment and reduction of systolic function and may also be associated with diastolic dysfunction [107]. An average PSI of all LV wall-segments of approximately 6% have been reported in patients with myocardial diseases such as hypertrophic cardiomyopathy and cardiac amyloidosis [112].

The relevance of PSS in myocardial disease has been questioned since it also occurs in healthy subjects [107]. PSS in healthy myocardium is suggested to have a small magnitude [111] and to be part of a normal and synchronized reshaping of the healthy ventricle, influenced by aging, sex , and loading conditions (increased with higher afterload and decreased with greater preload) [111]. Previous studies of healthy and low-risk middle aged populations have reported mean and median average PSI of all LV wall-segments of 1.2% and 2.0%, respectively [107, 113].

For both high-risk and low-risk populations, increasing average PSI of all LV wall-segments and increasing numbers of wall-segments exhibiting PSI >20% have been associated with increasing risk of cardiovascular morbidity and death [111, 113, 114].

7.2.2.3 Myocardial work

LV work can be derived from invasively measured pressure–volume loops, which describe the pressure-volume relationship and reflect myocardial oxygen consumption, and the LV performance [115, 116]. A novel, alternative non-invasive method for LV performance assessment is measure of myocardial work (MW) by analysis of pressure–strain loops [116]. This method incorporates analysis of non-invasively measured brachial artery blood pressure and LV deformation [116]. MW represents an index of LV work and is influenced by the LV loading conditions, the wall stress applied on the LV segments, and the contraction power of the myocardial fibres [116]. Analyses of non-invasively measured MW are found to strongly correlate

with invasively measured LV work [117, 118] and have been introduced as an advancement of LV strain analyses, due to reduction of the strain analyses` load dependent limitations [119, 120]. The clinical utility of MW is still questioned, but it is reported to add valuable information on LV function and prognosis in patients with cardiomyopathy and heart failure [116].

7.2.2.4 Left atrial longitudinal strain

Analyses of left atrial (LA) longitudinal strain are used to describe LA reservoir function during LV systole, conduit function during early diastole, and contraction during late diastole. LA reservoir and conduit strain are described as positive percentages as they represent lengthening of the myocardial fibres, while LA contraction strain during late diastole is described as negative percentages due to shortening of myocardial fibres. LA reservoir function measured by LA peak longitudinal strain has been reported to correlate well with invasively measured LV end diastolic pressure [121], to detect increased LV filling pressure at an earlier point than conventional echocardiographic diastolic parameters like LA volumes and mitral inflow and annular tissue velocities [122, 123], and to be of clinical and prognostic relevance especially concerning heart failure with preserved EF [124].

7.2.3 Cardiorespiratory fitness expressed by peak oxygen consumption

Cardiorespiratory fitness can be measured by a cardiopulmonary exercise test on a treadmill by standardized protocols and expressed as maximum VO_2 when a plateau VO_2 level is reached despite increasing workload, or by peak VO_2 at the highest observed VO_2 at maximal exhaustion. Maximum VO_2 is the highest rate at which oxygen can be taken up and utilized by the body during severe exercise and is normally mainly limited by the cardiac output [125]. The cardiac output is determined by stroke volume and heart rate, and reduced stroke volume is correlated to reduced myocardial function [126]. Even though maximum VO_2 is mainly limited by the ability of the cardiovascular system to deliver oxygen to the exercising muscles [125], other factors such as respiratory and musculoskeletal function, haemoglobin concentration, and deconditioning may also influence the result. Reduced cardiorespiratory fitness

and maximum VO_2 are associated with higher risk of cardiovascular disease and all-cause mortality, and improvement in cardiorespiratory fitness and maximum VO_2 are associated with reduced mortality risk [127].

8. Gaps in current knowledge

Preterm born

Previous studies of endothelial function in children and young adults born preterm or with low birthweight have presented findings of both normal and reduced endothelial function [20, 128-133]. However, only a few studies have included young adults born very or extremely preterm, or with extremely low birthweight, and studies of endothelial function assessed by FMD are rare in these groups. Since birthweight and GA are reported to be inversely associated with increased risk of cardiovascular disease [5, 6] assessment of endothelial function in these would add valuable knowledge and possibly contribute to improvements in risk stratification. Furthermore, even though register-based studies have reported increased risk of heart failure in adolescents and young adults born preterm [23, 26], clinical studies of LV myocardial function measured by myocardial resonance imaging or echocardiography have reported diverging results. Most former studies have found preserved LV systolic function measured by ejection fraction (EF) [134-136], but impaired diastolic function [137]. However, studies of strain, which allows detection of early stages of myocardial dysfunction, have reported reduced, normal, or even increased LV contractility in young adults born preterm [134-136, 138-140]. Recently, analyses of non-invasively measured MW [12, 11]and LA peak longitudinal strain [122, 123] have been introduced as advancements in the assessment of LV systolic and diastolic function. Analyses of myocardial function, including these new parameters, have to our knowledge not formerly been conducted in young adults born preterm or with low birthweight. The impact of birthweight and GA on LV systolic and diastolic myocardial function in adulthood is far from fully explored, and further studies including echocardiographic strain analyses would add valuable incremental information on this.

Paediatric survivors of childhood cancer

LV and RV myocardial function measured by strain and cardiorespiratory fitness have mainly been investigated in adult survivors of childhood cancer. Additionally, cardiovascular risk assessment and myocardial function by analysis of PSS in myocardial wall-segments have to our knowledge not formerly been evaluated in paediatric CCSs. However, assessment of myocardial function and cardiorespiratory fitness in paediatric CCSs is of importance to identify increased cardiovascular risk at an early point.

Date of completed literature study: May 13, 2023

9. Aims and objectives of the thesis

9.1 General aim

The general aim of this project was to expand the knowledge of cardiovascular health in populations with increased cardiovascular risk at young age due to severe disease and intensive treatment during development and growth; young adults born very preterm or with extremely low birthweight and paediatric survivors of childhood cancer.

9.2 Specific aims and objectives

Paper I: Investigate endothelial function by flow-mediated and nitroglycerine-induced vasodilatation of the brachial artery in young adults born very preterm or with extremely low birthweight.

Paper II: Investigate systolic and diastolic LV myocardial function by echocardiography, including measures of LV and LA strain and estimates of MW, in young adults born very preterm or with extremely low birthweight.

Paper III: Investigate systolic myocardial function and its association with cardiorespiratory fitness by echocardiographic measured LV and RV strain and cardiorespiratory exercise test in paediatric survivors of childhood cancer.

9.3 Research questions

Research question I: Do young adults born very preterm or with extremely low birthweight have reduced endothelial function assessed by flow-mediated and nitroglycerine-induced vasodilatation of the brachial artery compared to sex- and age matched controls?

Research question II: Do young adults born very preterm or with extremely low birthweight have reduced systolic and diastolic LV function measured by echocardiography, including assessment of LV and LA strain and estimates of MW, compared to sex- and age matched controls?

Research question III: Do paediatric survivors of childhood cancer have reduced LV and RV systolic myocardial function, assessed by echocardiographic measured LV and RV strain, compared to sex-and age matched controls? And how is their LV function associated with their maximal VO_2?

10 Material and methods

10.1 Study design and participants

The work presented in this thesis was based on observational studies designed as population-based cohort studies; the single centre study “Project Extreme Prematurity” (PEP) and the multicentre study “Physical Activity and fitness in Childhood Cancer Survivors” (PACCS). Results from the PEP study are described in paper I and II, while results from the PACCS study are described in paper III.

10.1.1 PEP

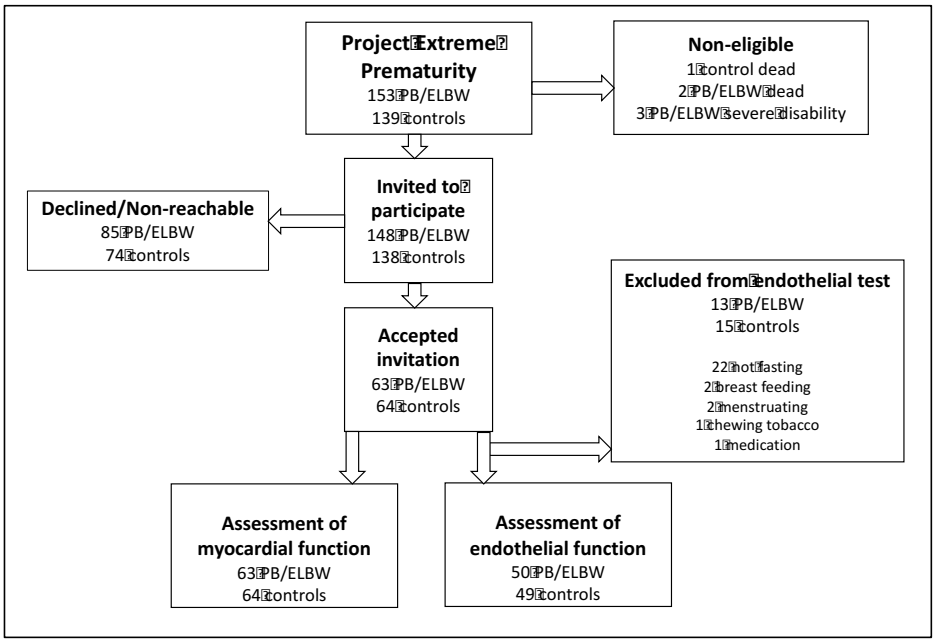
The Project Extreme Prematurity led by the WestPaed Research group affiliated to Haukeland University Hospital in Bergen and the University of Bergen, Norway, has included three cohorts of young adults born very preterm with GA <29 weeks or with extremely low birthweight <1000 grams (PB/ELBW) and individually age-and sex matched term-born controls from Western Norway. The term-born controls were identified using birth protocols from the maternity ward and each PB/ELBW was matched with the next-born child with similar sex, GA >37 weeks, and birthweight >3000 grams.

The subjects born in 1982-1985 (cohort 1) and in 1991-1992 (cohort 2) were retrospectively recruited, and those born in 1999-2000 (cohort 3) were prospectively recruited, in total 153 PB/ELBW and 139 controls. Of these, 6 individuals were lost to follow-up; 1 control and 2 PB/ELBW because of death and 3 PB/ELBW due to severe disability. The participants have been followed longitudinally since year 2000 and were previously examined in year 2001 and 2008.

During the period from November 2017 to February 2020 individuals from the three cohorts were invited to a third follow-up, including a first-time ultrasound-based examination of endothelial function and examination of myocardial function by echocardiography. Subjects who were not fasting, were menstruating or breast feeding, or used vasoactive medication or nicotine were excluded from the endothelial function test. Endothelial function was assessed in a total of 99 participants including 50 PB/ELBW (48% biological males) and 49 controls (41% biological males). Myocardial function was assessed in a total of 127 participants,

including 63 PB/ELBW (43% biological males) and 64 controls (44% biological males) (Figure 4). The characteristics of the participants by cohort are described in Table 4.

Figure 4 Flow chart of participants in the PEP study [1, 2]



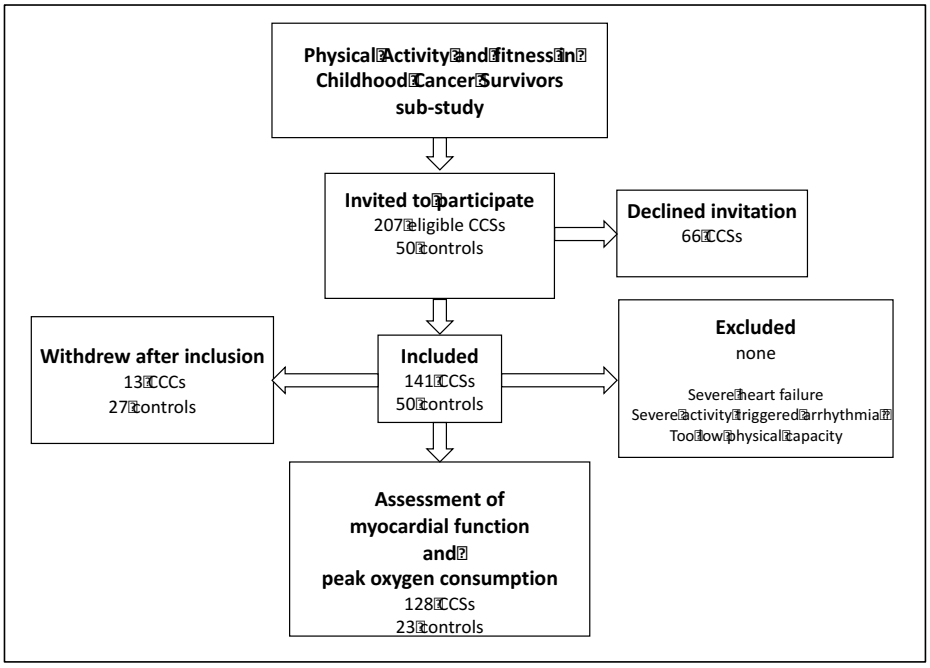
10.1.2 PACCS

The study "Physical Activity and fitness in Childhood Cancer Survivors" [141] is an international multicentre study involving Norway (Oslo and Bergen), Denmark (Copenhagen), Switzerland (Basel), Finland (Turku and Tampere), and Germany (Essen) with main aims to identify levels of and mediators of activity and physical fitness in CCSs. The PACCS study comprises four work packages. Participants from work package 1 were recruited to participate in work package 2 (and, with time, work packages 3 and 4).

The present national sub-study was part of work package 2 and planned recruitment of 150 CCSs from work package 1 and 50 controls. The main aim of the sub-study was to investigate myocardial function and physical fitness in CCSs assessed by echocardiography and cardiopulmonary exercise test performed on a treadmill. CCSs

who were attending routine follow-ups at paediatric cancer outpatient clinics at Oslo University Hospitals and Haukeland University Hospital (Bergen) in Norway were invited to participate. To qualify for inclusion, they were aged 9-18 years, and the cancer treatment had finished at least one year before. Exclusion criteria, based on feasibility of the cardiopulmonary exercise test, included severe heart failure with echocardiographic measured fraction shortening below 20%, severe activity triggered arrhythmia, or level of physical function too low to enable completion of the exercise test. None of the invited participants were excluded due to this. Healthy age- and sex-matched schoolmates or friends were recruited as control participants by the CCSs. Because of capacity challenges regarding echocardiographic imaging only one center, Haukeland University Hospital, recruited control participants. Additionally, examination of controls was limited by the ongoing COVID-19 pandemic due to restrictions regarding travelling and entrance to hospitals and frequent COVID-quarantine in school children. During the period from February 2019 to February 2021, a total of 128 CCSs (52% biological males) and 23 controls (44% biological males) underwent an echocardiographic examination and a cardiopulmonary exercise test (Figure 5).

Figure 5 Flow chart of participants in the PACCS sub-study [142]



10.2 Ethical aspects

The studies were conducted in accordance with the Helsinki declaration. The Regional Committee for Medical and Health Research Ethics of the Western Norway Health Authority approved the PEP study described in paper I and II (REC 2017/628), and the Regional Committee for Medical and Health Research Ethics of the South-East Norway Health Authority approved the PACCS study described in paper III (REC 2018/739). Age adapted verbal and written information was given to the participants. The studies presented here relied on non-invasive tests. Hence, there was low risk, with minimal inconveniences to the participants and their guardians. Children were offered dermal application of a local anaesthetic (Emla®) prior to blood sample collections to minimize pain.

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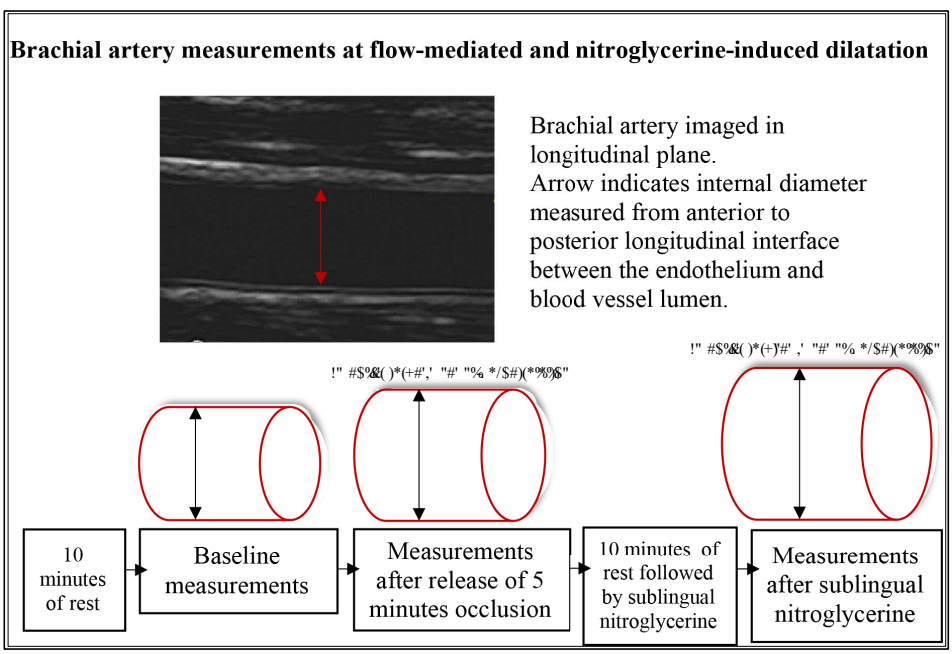

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10.4 Echocardiographic measures of systolic and diastolic myocardial function

All echocardiographic examinations were performed with Vivid E9 ultrasound system (GE Healthcare, Horten, Norway) using a 5Sc (1.5-4.6 MHz) or equivalent 4Vc-D (1.4-5.2 MHz) transducer for all imaging, and stored for offline analyses by the echocardiographic software, EchoPAC version 204 (GE Healthcare, Horten, Norway). All participants underwent a comprehensive functional echocardiographic examination, including grayscale images optimized for 2-dimensional speckle analysis. Speckle-tracing was performed using automated function imaging.
All imaging and analyses were done according to recommendations from the European and American societies of echocardiography [143-146], and as proposed by Russell et al. [118] regarding estimates of MW.

10.4.1 Left and right systolic myocardial function

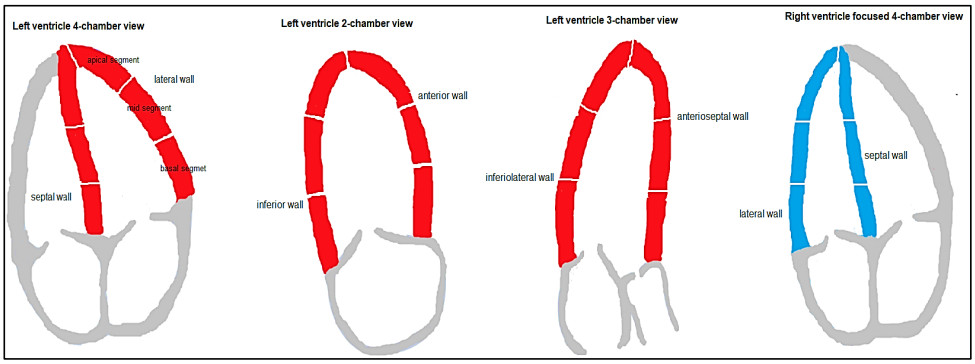
10.4.1.1 Ejection fraction

LV EF was measured by Simpson apical biplane technique.

10.4.1.2 Left and right ventricular longitudinal strain

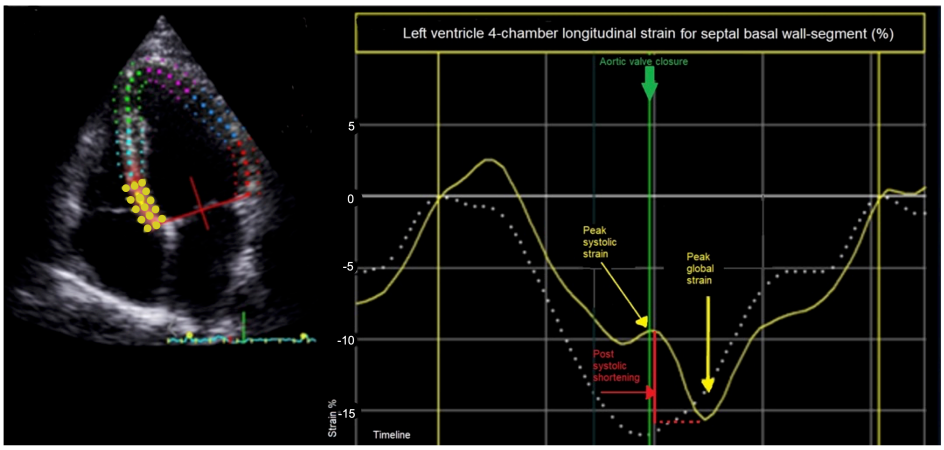
Gray scale images were acquired at frame rate (frames/second) to heart rate (beats/minute) ratio 0.7-0.9 for speckle-tracing analysis. The region of interest included the endocardium and myocardium from the atrioventricular valve annulus to the apex. Longitudinal strain curves from ventricular cycles from standard apical four, three, and two chamber view images, analysed for 18 sub-segments, were used for LV global longitudinal strain (GLS) assessments, and longitudinal strain curves from a RV-focused four chamber view image, analysed for 6 sub-segments including the interventricular septum, were used for RV longitudinal strain assessments (Figure 7). Peak systolic longitudinal strain was measured at aortic and pulmonary valve closure for LV and RV peak longitudinal strain measures, respectively.

Figure 7 Illustration of left and right ventricular images, including walls and wall-segments [142]



Delayed myocardial contraction after aortic and pulmonary valve closure, PSS, was measured by PSI (Figure 8). PSS was assessed by determination of the presence or absence of any degree of PSS (defined as PSI >0%) in the myocardial wall-segment, and by measures of mean PSI of wall-segments, walls, and the whole ventricle (global-PSI).

Figure 8 Post systolic shortening measured by post systolic index [142]



Speckle-tracking derived longitudinal strain of basal septal wall-segment (yellow line) in left ventricle 4-chamber image. Post systolic shortening (red line) is defined as late systolic longitudinal shortening appearing after the aortic valve closure (green line) in one cardiac cycle and is calculated as post systolic index:

$$\text{PSI} = \frac{(\text{peak global strain} - \text{peak systolic strain})}{(\text{peak global strain})} \text{ x } 100\%$$

In this example:

$$\text{PSI} = \frac{(-15\%) - (-10\%)}{(-15\%)} \text{ x } 100\% = 33\%$$

The dashed white line represents longitudinal strain of a wall-segment without post systolic shortening.

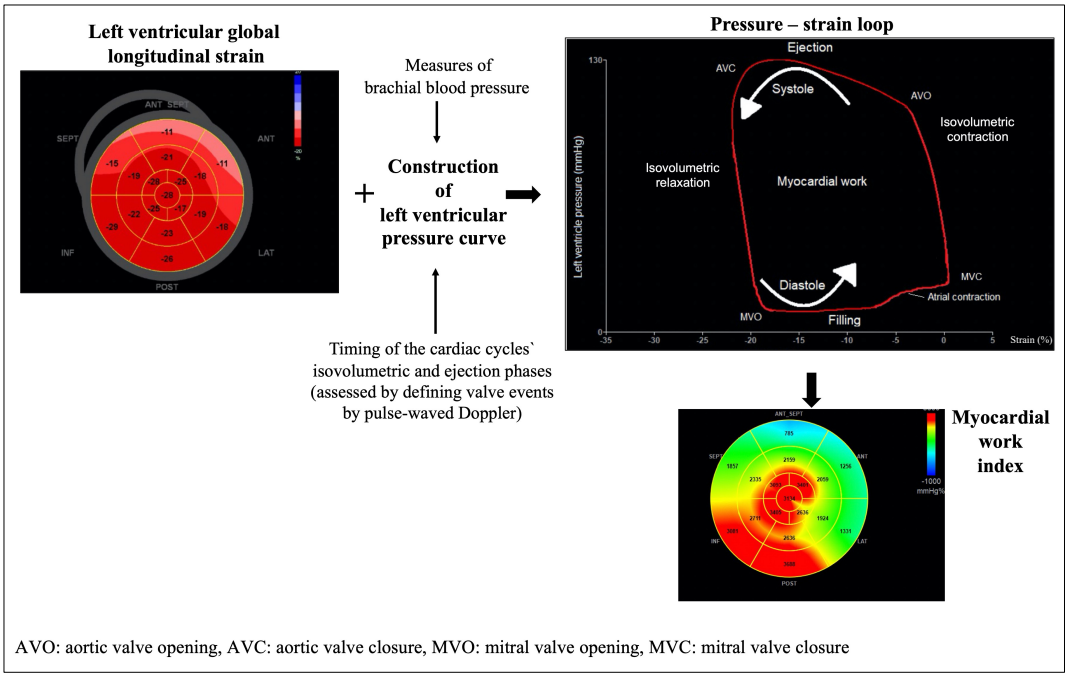
In all paediatric study participants (paper III), LV longitudinal strain z-scores were calculated as proposed by Dallaire et al. [147] from measurements in apical four chamber view images, normalized to body surface area (BSA) and adjusted for heteroscedasticity. The z-score calculation was obtained from the equation:

$$Z = \frac{(\text{observed value} - 20.295 \times \text{BSA}^{0.5})}{(-0.343 \times \text{BSA} + 2.02)}$$

10.4.1.3 Myocardial work

Estimates of MW were calculated from a complex algorithm by the EchoPAC software after determination of LV peak systolic global longitudinal strain (LV-GLS) and construction of a LV pressure curve (derived from non-invasive brachial blood pressure measures and the timing of the cardiac cycles` isovolumetric and ejection phases, which were defined by opening and closure of the mitral valve by pulse-waved Doppler). The area of the LV pressure-strain loop represents MW (Figure 9).

Figure 9 Estimates of myocardial work by pressure-strain loop [2]



AVO: aortic valve opening, AVC: aortic valve closure, MVO: mitral valve opening, MVC: mitral valve closure

Quantification of MW was described in the following terms:

- Global work index (GWI): describes total amount of LV work performed during systole from the opening to the closure of the mitral valve.
- Myocardial global constructive work (GCW): describes work that contributes to ejection (positive LV work (shortening) during systole plus negative work (lengthening) during isovolumetric relaxation).

- Myocardial global wasted work (GWW): describes work that does not contribute to ejection (negative LV work during systole plus positive work during isovolumetric relaxation).
- Global work efficiency (GWE): describes global constructive work relative to the total of global constructive and wasted work.

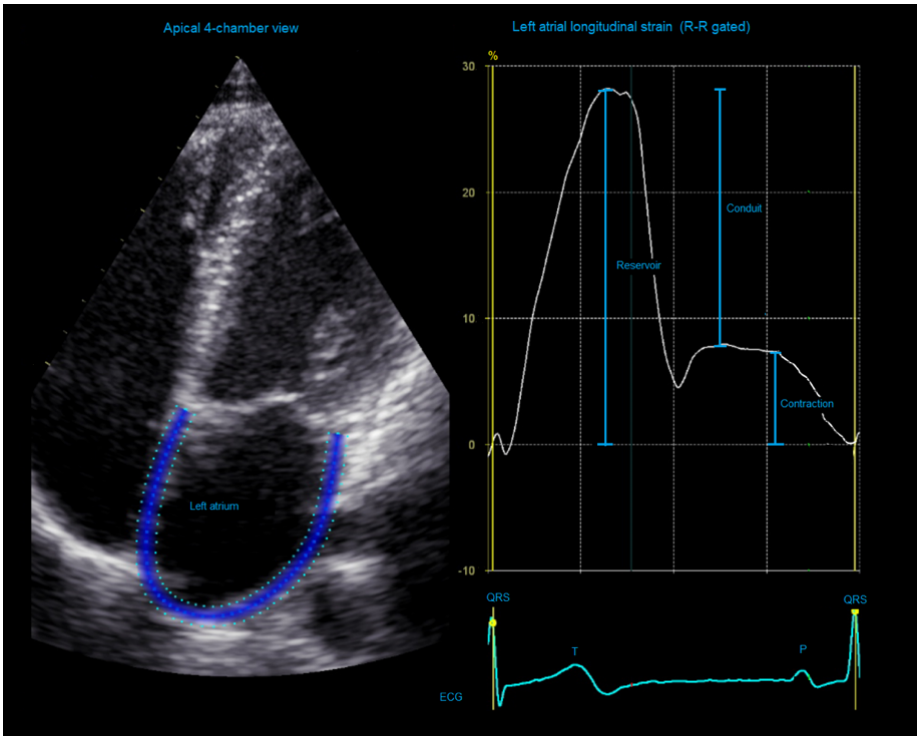
10.4.2 Diastolic myocardial function

Determination of the presence or absence of elevated LV filling pressure was measured by:

- Early/late mitral inflow velocity (E/A) (pulse waved Doppler)
- Mitral septal and lateral annular early diastolic velocity (e´) and E/e´ (continuous wave Doppler)
- Tricuspid valve regurgitation jet peak gradient (continuous wave Doppler)
- LA end-systolic volume indexed by body surface area (four chamber view images only)
- LA reservoir strain (peak longitudinal strain), conduit strain, and contractile strain

Longitudinal strain curves from atrial cycle from apical four chamber view, with zero reference point set at the start of the ECG R-wave were used for LA longitudinal strain assessment. Peak systolic longitudinal strain was measured at end-systole for LA measures (Figure 10).

Figure 10 Left atrial longitudinal strain [2]



10.5 Cardiopulmonary exercise test

Cardiopulmonary exercise test was performed by walking/running on a treadmill (Woodway PPS MED, WOODWAY USA, Waukesha, WI, USA) with an incremental ramp protocol until exhaustion. The starting workload was at 0% incline with increasing speed from 3.0 until 4.0 km/hour the first 2 minutes. Further, the workload was increased every second minute; the inclination by 2% and the speed by 1 km/hour, respectively. If the participant continued for more than 10 minutes, the inclination was increased by 1.5% to keep the increase in workload approximately equal at each step (15-20 Watt/step). The participant was breathing into a Hans Rudolph two way breathing mask (2700 series; Hans Rudolph Inc, Kansas City, USA) connected to an OxyconPro analyser (Jaeger, Würtzburg, Germany) using the breath-by-breath method. Borg scale [148] (scale 6-20) was used as rating of perceived exertion immediately after the test. The highest oxygen uptake measured, which

remained stable over at least 30 seconds, was used as peak VO_2 if two of the following three criteria were fulfilled: Respiratory gas exchange ratio ≥1.10; Borg scale ≥17; or VO_2 reach a plateau with increasing workload.

10.6 Statistical analyses

Descriptive variables were presented as numbers and percentages and outcome data as means with 95% confidence interval (CI) or standard deviation (SD). The Kolmogorov- Smirnov test was used to test the normality of the outcome data.

For comparison of descriptive and outcome data (without significant confounders) between two groups (cases and controls) Chi-square test or Fisher`s exact test, independent samples t-test (with equal variance not assumed) or two samples Kolmogorov-Smirnov test were applied, as appropriate. For comparison of descriptive and outcome data between more than two groups (subgroups) Chi-square test or Fisher`s exact test, classic one-way ANOVA or Welch´s ANOVA (with Tukey`s test and Games-Howell used for post hoc analyses, respectively) were applied, as appropriate.

Linear regression analyses were used to identify confounders. Analyses of covariance (ANCOVA) were applied for comparison of data between two groups, and multiple comparison analyses (with Sidak correction) were applied for comparison of data between more than two groups.

To investigate whether the difference in echocardiographic measures between groups differed by sex, an interaction term for sex and group affiliation was added.

Pearson correlation coefficient (PCC) was used to explore associations between outcome data and descriptive variables.

During analyses in the PEP study, GA in controls was set to 40 weeks.

Inter- and intra-rater reliability were measured by a 2-way mixed-effect model for absolute agreement.

All tests were two-sided, and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

11. Summary of results

11.1 PEP

The PB/ELBW group and control group were well matched for several known cardiovascular risk factors. The PB/ELBW had significantly lower mean BSA compared to the controls, otherwise, there were no significant differences for chronological age, body mass index (BMI), systolic and diastolic blood pressure, HbA1c level (measured in 78% and 73% of PB/ELBW and in 96% and 95% of controls in paper I and II, respectively) or smoking status (4%). Additionally, none of the participants had history of cardiovascular disease. For the PB/ELBW, the mean age at examination was 28 years (SD 6) and 27 years (SD 6), mean GA was 27 weeks (SD 2), and mean birthweight 961 g (SD 225) and 945 g (SD 217) in paper I and II, respectively.

The PB/ELBW in the youngest cohort (born in year 1999-2000) had the lowest mean birthweight and the largest share of participants born extremely preterm (GA<28 weeks) and with extremely low birthweight (<1000 g). Additionally, they were more frequently treated with surfactant, steroids, and continuous positive airway pressure in the neonatal period (Table 4).

11.1.1 Endothelial function

Mean absolute FMD, relative FMD, and endothelial-dependent dilatation capacity (FMD relative to maximal NID) were reduced in the PB/ELBW compared to controls, 0.17 mm (95% CI 0.14, 0.21) versus 0.24 mm (95% CI 0.20, 0.28) (p= 0.01), 5.4% (95% CI 4.2, 6.6) versus 7.5% (95% CI 6.2, 8.9) (p= 0.02), and 20.1% (95% CI 16.1, 24.5) versus 30.8% (95% CI 25.9, 35.6) (p= 0.001), respectively (Figure 11 and Table 5).

Interaction analyses did not show a significant sex effect on the total group difference in the measures of endothelial function.

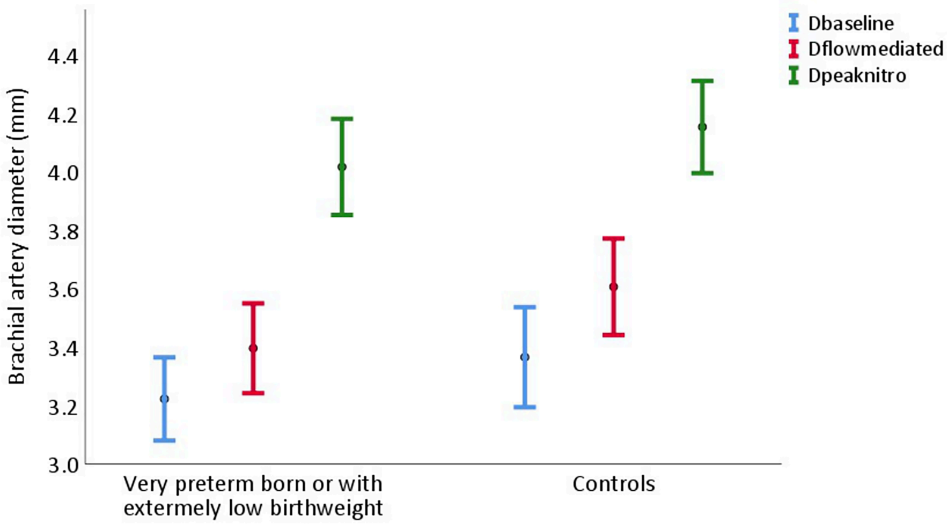
Correlation analyses, which included PB/ELBW and controls, revealed a significant positive association between birthweight and FMD (PCC 0.3), birthweight and endothelial-dependent dilatation capacity (PCC 0.3), GA and FMD (PCC 0.2), and GA and endothelial-dependent dilatation capacity (PCC 0.3). However, in analyses of

differences between the three cohorts of PB/ELBW, we found no difference in FMD or endothelial-dependent dilatation capacity between cohorts (Table 4).

Correlation analyses revealed no significant associations between treatment in the prenatal and neonatal periods (days of oxygen supplement, days of ventilation support, use of surfactant, or steroid treatment) and measures of endothelial function.

Figure 11 [1]

Flow-mediated and nitroglycerine induced dilatation of the brachial artery



Brachial artery diameter at baseline (blue), after flow-mediated dilatation (red), and after nitroglycerine induced dilatation (green) in PB/ELBW and term-born controls, presented as mean with 95% confidence interval (95% CI).
Dbaseline: brachial artery diameter at baseline (blue); Dflowmediated: brachial artery diameter after flow-mediated dilatation (red); Dpeaknitro: brachial artery diameter after nitroglycerine induced dilatation (green).
Absolute flow-mediated dilatation was 0.17 mm (95% CI 0.14, 0.21) for PB/ELBW and 0.24 mm (95% CI 0.20, 0.28) for controls. Percentage FMD was 5.4% (95% CI 4.2, 6.6) for PB/ELBW and 7.5% (95% CI 6.2, 8.9) for controls. Endothelial-dependent capacity was 20.1% (95% CI 16.1, 24.5) for PB/ELBW and 30.8% (95% CI 25.9, 35.6) for controls.

11.1.2 Left ventricular systolic myocardial function

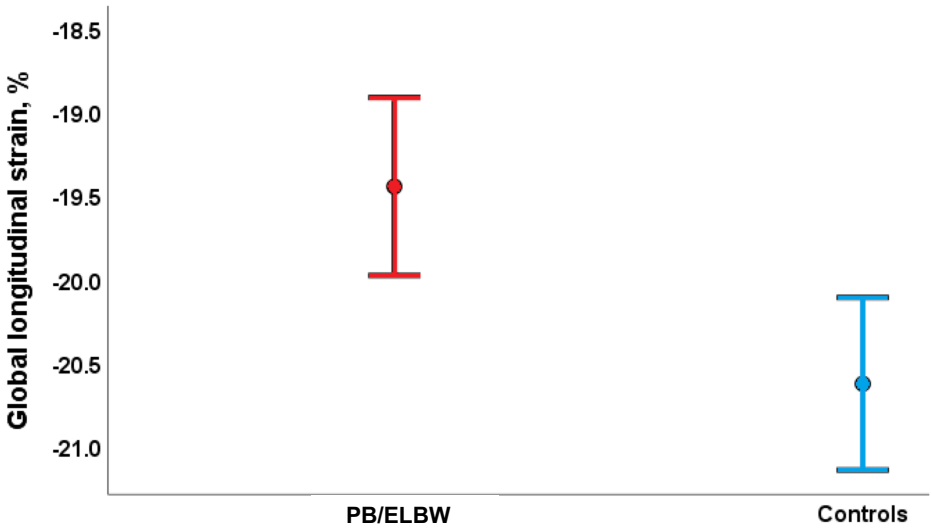
Mean LV-GLS was reduced in PB/ELBW compared to controls, -19.4% (95% CI -20.0, -18.9) versus -20.6% (95% CI -21.1, -20.1) (p= 0.003), respectively (Table 5 and Figure 12), and 6% of the PB/ELBW had impaired systolic myocardial function measured by EF Simpson (<50%) or LV-GLS (>-16%). Measures of MW were similar in PB/ELBW and controls (Table 5).

Interaction analyses did not show a significant sex effect on the total group difference in LV-GLS between PB/ELBW and controls.

Correlation analyses, which included PB/ELBW and controls, revealed that lower birthweight, but not GA, was significantly associated with lower myocardial function measured by LV-GLS (PCC -0.2). However, in analyses of differences between the three cohorts of PB/ELBW, we found no difference in measures of systolic myocardial function between the cohorts (Table 4).

Correlation analyses revealed no significant associations between treatment in the prenatal and neonatal periods (days of oxygen supplement, days of ventilation support, use of surfactant, or steroid treatment) and measures of systolic myocardial function.

Figure 12 [2]



Left ventricular myocardial function measured by peak global longitudinal strain (GLS) was significantly reduced in young adults born very premature or with extremely low birthweight (PB/ELBW) compared to controls. Mean GLS was -19.4% (95% confidence interval (CI) -20.0, -18.9) in PB/ELBW and -20.6% (95% CI -21.1, - 20.1) in controls (p = 0.003).

11.1.3 Left ventricular diastolic myocardial function

Measures of diastolic function, including LA reservoir strain, were mainly within normal range and similar in PB/ELBW and controls (Table 5).

Correlation analyses, including the PB/ELBW and controls, revealed that increasing chronological age, but not birthweight or GA, was associated with decreasing diastolic function measured by LA conduit strain, E/e´, and mitral septal and lateral e´ (PCC -0.2, PCC 0.3, PCC -0.5, and PCC -0.2, respectively). However, in analyses of differences between the three cohorts of PB/ELBW, we found no difference in measures of diastolic myocardial function between the cohorts (Table 4).

Correlation analyses revealed no significant associations between treatment in the prenatal and neonatal periods (days of oxygen supplement, days of ventilation support, use of surfactant, or steroid treatment) and measures of diastolic function.

Table 4 Characteristics, echocardiographic measurements, and measures of endothelial function of young adults born very preterm or with extremely low birthweight by cohort [1, 2]

	Cohort I born 1982-85 n=23	**Cohort II** born 1991-92 n=19	**Cohort III** born 1999-2000 n=21	p
Characteristics				
Age (years), mean (SD)	34 (1.5)	27 (0.6)	19 (0.8)	<0.001
BW (grams), mean (SD)	1014 (210)	959 (225)	857 (194)	0.05
GA (weeks), mean (SD)	28 (2)	27 (2)	27 (1)	0.1
Extremely preterm GA <28 weeks, n (%)	11 (48%)	11 (58%)	17 (81%)	0.1
Extremely BW <1000 g, n (%)	9 (39%)	11 (58%)	17 (81%)	0.02
Moderate/severe BPD, n (%)	6 (26%)	8 (42%)	7 (33%)	0.6
Days O_2-supplement, mean (SD)	44 (21)	61 (51)	53 (25)	0.3
Days on ventilator, mean (SD)	10 (10)	8 (10)	6 (5)	0.3
Days on CPAP, mean (SD)	0	0	33 (14)	
Surfactant, n (%)	0	9 (47%)	19 (90%)	<0.001
Pre-natal steroid treatment, n (%)	8 (35%)	7 (37%)	20 (95%)	<0.001
Post-natal steroid treatment, n (%)	1 (4%)	6 (3%)	7 (33%)	0.03
Echocardiographic measurements				
EF Simpson (%), mean (95% CI)	60 (57, 61)	60 (57,63)	59 (56, 62)	0.6
LV-GLS (%), mean (95% CI)	-20.2 (-21.0, -19.5)	-20.0 (-20.7, -19.3)	-19.9 (-20.8, -19.1)	0.9
GWI (mmHg%), mean (95% CI)	1667 (1484, 1849)	1847 (1673, 2020)	1709 (1504, 1913)	0.3
Average mitral E/e´ratio, mean (95% CI)	5.6 (5.2, 5.9)	5.7 (5.2, 6.2)	5.2 (4.9, 5.7)	0.4
Mitral septal e´ (m/s), mean (95% CI)	0.10 (0.09, 0.11)	0.11 (0.10, 0.13)	0.13 (0.11, 0.12)	0.001
Mitral lateral e´(m/s), mean (95% CI)	0.14 (0.12, 0.15)	0.15 (0.13, 0.16)	0.17 (0.15, 0.18)	0.012
LA reservoir strain (%), mean (95% CI)	38 (33, 43)	38 (33, 43)	38 (33, 44)	1.0
LA conduit strain (%), mean (95% CI)	26 (22, 30)	27 (22, 31)	30 (25, 34)	0.55
LA volume (ml/m2), mean (95% CI)	22 (19, 25)	21 (18, 24)	20 (17, 23)	0.6
Endothelial function	n =21	n= 16	n= 13	
Absolute FMD (mm), mean (95% CI)	0.20 (0.14, 0.26)	0.16 (0.08, 0.23)	0.16 (0.08, 0.25)	0.6
Percentage FMD (%), mean (95% CI)	5.8 (4.0, 7.7)	5.0 (2.6, 7.4)	5.2 (2.3, 8.2)	0.8
FMD/NID (%), mean (95% CI)	24.1 (17.1, 31.2)	16.4 (9.7, 23.4)	18.5 (9.4, 27.7)	0.3

SD: standard deviation, BW: birthweight, GA: gestational age, BPD: bronchopulmonary dysplasia (classified as moderate/severe BPD if need of supplementary oxygen or CPAP at 36 weeks of gestation), O_2: oxygen, CPAP: continuous positive airway pressure, EF: ejection fraction, CI: confidence interval, LV-GLS: left ventricle global longitudinal strain, GWI: global work index, LA: left atrium, FMD: flow-mediated dilatation, NID: nitroglycerine induced dilatation

Table 5 Measures of endothelial and myocardial function in young adults born very preterm or with extremely low birthweight and controls [1, 2]

	PB/ELBW		Controls		P
	n	mean (95% CI)	n	mean (95% CI)	
Endothelial function					
Absolute FMD (mm)	50	0.17 (0.14, 0.21)	49	0.24 (0.20, 0.28)	0.01
Percentage FMD (%)	50	5.4 (4.2, 6.6)	49	7.5 (6.2, 8.9)	0.02
FMD/NID (%)	50	20.3 (16.1, 24.5)	49	30.8 (25.9, 35.6)	0.001
Systolic myocardial function					
EF Simpson (%)	63	59 (58, 61)	64	61 (59, 62)	0.32
LV-GLS (%)	50	-19.4 (-20.0, -18.9)	53	-20.6 (-21.1, -20.1)	0.003
GCW (mmHg%)	49	1982 (1901, 2063)	51	2074 (1995, 2153)	0.11
GWW (mmHg%)	49	100 (78, 121)	51	92 (71, 113)	0.61
GWI (mmHg%)	49	1749 (1666, 1832)	51	1853 (1772, 1934)	0.08
GWE (%)	49	95 (93, 96)	51	95 (93, 96)	0.85
Diastolic myocardial function					
Mitral septal e´(m/s)	61	0.12 (0.11, 0.12)	63	0.12 (0.11, 0.13)	0.99
Mitral lateral e´ (m/s)	62	0.15 (0.14, 0.16)	64	0.16 (0.13, 0.20)	0.96
Average mitral E/e´ratio	61	5.5 (5.3, 5.7)	61	5.4 (5.1, 5.8)	0.82
Mitral E/A-ratio	63	1.5 (1.4, 1.6)	61	1.5 (1.4, 1.6)	0.83
TR peak gradient (mmHg)	37	18 (16, 20)	41	16 (15,18)	0.96
LA contraction strain (%)	55	-10.5 (-11.5, -9.4)	55	-11.5 (-12.5, -10.3)	0.22
LA conduit strain (%)	55	27.5 (25.5, 29.5)	55	28.6 (26.6, 30.6)	0.43
LA reservoir strain (%)	55	38.1 (35.7, 40.5)	55	39.7 (37.4, 42.1)	0.34
LA volume (ml/m^2)	55	21 (19, 22)	55	21 (19, 22)	0.93

PB/ELBW: very premature born (< 29 weeks of gestation) or with extremely low birthweight (<1000 g), CI: confidence interval, FMD flow-mediated dilatation: NID nitroglycerine induced dilatation: EF: ejection fraction, LV: left ventricle, GLS: global longitudinal strain, GCW: global constructive work, GWW: global wasted work, GWI: global work index, GWE: global work efficiency, TR: tricuspid regurgitation, LA: left atrium

11.2 PACCS

Acute leukaemia (48%), cerebrospinal neoplasms (13%), and renal tumours (11%) were the most frequent diagnoses in our CCS population, and 97% and 5% had received chemotherapy and radiation therapy involving the chest, respectively. Mean anthracycline dose was 152 mg/m^2 (SD 91). Four CCSs had elevated NT-proBNP values [149], and mean NT-proBNP was 67 ng/L (SD 48). The CCSs and controls were similar regarding sex and ethnicity distribution, and regarding estimated means of height, weight, BSA, BMI, and diastolic blood pressure. The mean systolic blood pressure was lower in the CCSs (107 mmHg (95% CI 105, 108)) compared to the controls (112 mmHg (95% CI 108, 116)) (p= 0.03) and mean age was higher in the CCSs (13.6 years (SD 2.6)) compared to the control group (12.7 years (SD 3.1)) (p= 0.04). (Table 6)

11.2.1 Left and right ventricular function measured by longitudinal strain

LV-GLS was reduced in the CCSs compared to the controls, -19.7% (95% CI -20.1, -19.3) versus -21.3% (95% CI -22.2, -20.3) (p= 0.004), respectively (Table 6 and Figure 13). This also applied for the anthracycline naive participants and the subgroup of CCS treated with low dose of anthracycline (<100 mg/m^2) which both had lower LV longitudinal strain z-score compared to the control group (-0.1 (95% CI -0.6, 0.4) and -0.1 (95% CI -0.4, 0.5), respectively, versus 0.9 (95% CI 0.2, 1.5), both p <0.02) (Figure 14). Of the CCS 13% had reduced LV longitudinal strain z-score (<-2).

Right ventricular longitudinal strain (RV-LS) was similar in CCSs and controls (Table 6).

The presence of any degree of PSS (PSI >0%) was found more frequently in CCSs wall-segments compared to wall-segments in the controls; 41% versus 33% (p= 0.003) of LV wall-segments and 30% versus 21% (p= 0.04) of RV wall-segments in CCSs and controls, respectively. Mean global PSI values for the left and right ventricle were similar in CCSs and controls (Table 6).

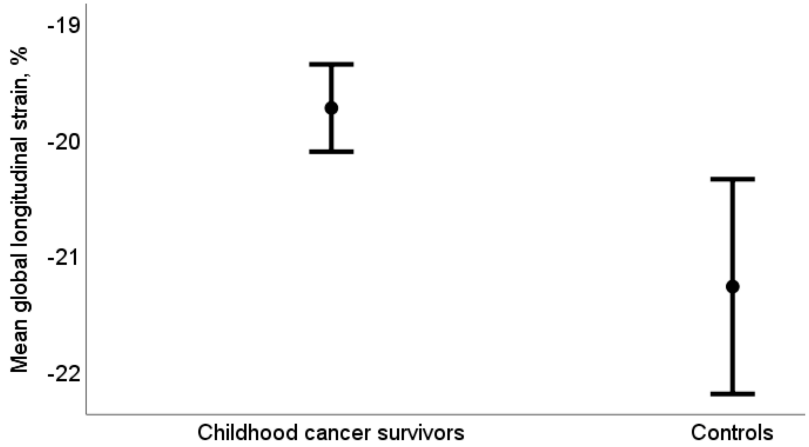
Table 6 Characteristics, measures of myocardial function, and measurements of peak oxygen consumption in paediatric childhood cancer survivors and controls [142]

	CCS		Controls		p
	n		n		
Age at study, years, mean (SD)	128	13.6 (2.6)	23	12.7 (3.1)	0.04
Sex, males	66	52%	10	44%	0.48
Ethnicity, Caucasian Ethnicity, Asian Ethnicity, mixed Ethnicity, other	117 3 7 1	91% 2% 6% 1%	23	100%	0.79
Height, cm, mean (95% CI)	128	158 (155, 160)	23	153 (147, 158)	0.11
Weight, kg, mean (SD)	128	51 (15)	23	45 (14)	0.08
BMI, kg/m^2, mean (95% CI)	128	20.2 (19.6, 20.9)	23	19.0 (17.5, 20.5)	0.14
BSA, m^2, mean (95% CI)	128	1.5 (1.4, 1.5)	23	1.4 (1.3, 1.5)	0.09
Systolic BP, mmHg, mean (95% CI)	128	107 (105, 108)	23	112 (108, 116)	0.03
Diastolic BP, mmHg, mean (95% CI)	128	65 (64, 67)	23	69 (65, 73)	0.07
Age at diagnosis, years, mean (SD)	128	5.3 (3.5)	23		
Time since diagnosis, years, mean (SD)	128	8.3 (3.7)	23		
Time after last treatment, years, mean (SD)	128	6.6 (3.6)	23		
LV-EF Simpson, %, mean (95% CI)	128	60 (59, 61)	23	63 (61, 66)	0.01
Z-score LV-LS, mean (95% CI)	126	-0.3 (-0.6, -0.1)	23	0.9 (0.2, 1.5)	0.001
LV- GLS, %, mean (95% CI)	118	-19.7 (-20.1, -19.3)	22	-21.3 (-22.2, -20.3)	0.004
PSI-global LV, %, mean (95% CI)	104	2.1 (1.5, 2.4)	21	1.8 (1.0, 2.5)	0.35
LV -segments with PSI > 0%, %	126	41%	22	33%	0.003
RV-LS, %, mean (95% CI)	121	-23.2 (-23.7, -22.6)	23	-23.3 (-24.6, -22.0)	0.84
PSI-global RV, %, mean (95% CI)	103	1.8 (1.2, 2.4)	18	2.3 (0.7, 3.8)	0.59
RV-segments with PSI > 0%, %	120	30%	21	21%	0.04
Peak VO_2, ml/kg/min, mean (95% CI)	126	43.2 (41.4, 44.9)	22	48.6 (44.5, 52.6)	0.01

CCS: childhood cancer survivor, SD: standard deviation, CI: confidence interval, BMI: body mass index, BSA: body surface area, BP: blood pressure, LV-EF: left ventricular ejection fraction, LV-LS: left ventricular longitudinal strain, LV-GLS: left ventricular global longitudinal strain, PSI: post systolic index, RV-LS: right ventricular longitudinal strain, VO_2: oxygen consumption

Figure 13 [142]

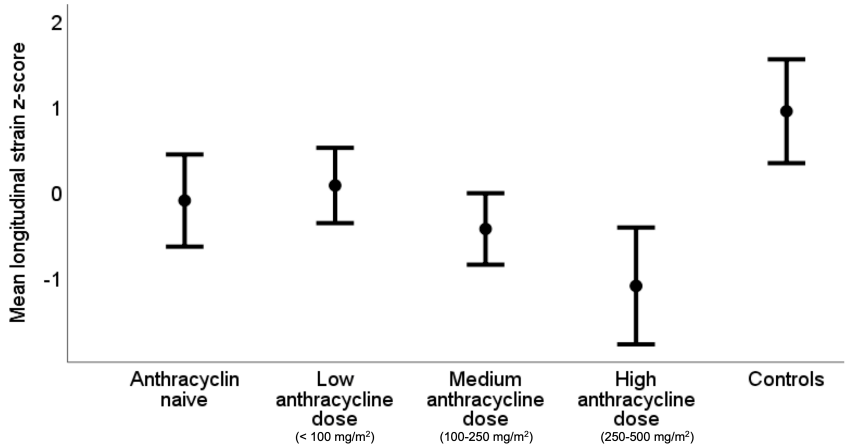
Left ventricle global longitudinal strain in childhood cancer survivors and controls



Mean left ventricular global longitudinal strain was -19.7% (95% confidence interval (CI) -20.1, -19.3) for childhood cancer survivors (n = 118) and -21.3% (95 % CI -22.2, -20.3) for controls (n = 22) (p = 0.004).

Figure 14 [142]

Left ventricle longitudinal strain z-score in subgroups of childhood cancer survivors and controls



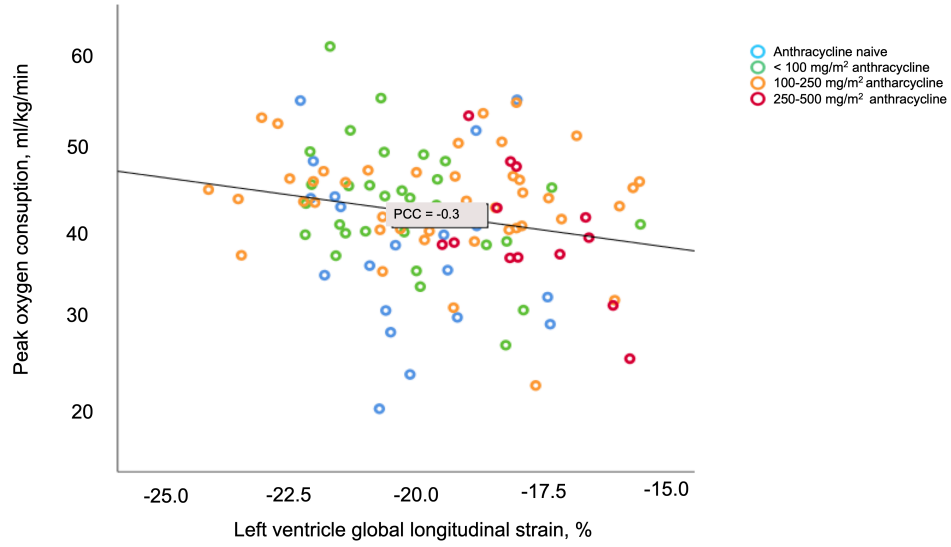
Mean left ventricular longitudinal strain z-score (LV-LS z-score) was impaired in all of the 4 childhood cancer survivor subgroups compared to the control group (all p-values ≤ 0.02). For the anthracycline naive subgroup and the subgroups treated with low, medium, and high dose of anthracyclines z-score was -0.1 (95% confidence interval (CI) -0.6, 0.4), 0.1 (95% CI -0.4, 0.5), -0.4 (95% CI -0.8, -0.02), and -1.1 (95% CI -1.8, -0.4), respectively, versus 0.9 (95% CI 0.2, 1.5) in the controls.

11.2.3 Correlation analyses between myocardial function and time, treatment, and peak VO_2

Among the CCSs, lower LV myocardial function was associated with lower peak VO_2 (PCC= -0.3 for LV-GLS) (Figure 15) and higher dose of anthracycline and increasing time after treatment were associated with lower left and right ventricular myocardial function (PCC= 0.5 for LV-GLS and 0.2 for RV-LS and PCC= 0.3 for LV-GLS and 0.2 for RV-LS, respectively). We found no significant associations between myocardial function and age at diagnosis, NT-proBNP levels, or radiation therapy to the chest.

Figure 15 [142]

Peak oxygen consumption and left ventricular global longitudinal strain in childhood cancer survivors



Decreasing myocardial function measured by left ventricular global longitudinal strain was associated with decreasing peak oxygen consumption in childhood cancer survivors (Pearson correlation coefficient (PCC) -0.3, p = 0.01).

12. Discussion

12.1 Discussion of study design and study populations

The work presented in this thesis was based on observational studies which aimed to find true associations between exposure and outcome in the study populations that could be generalizable to the populations the study samples were intended to represent; young adults born very preterm or with extremely low birthweight (paper I and II) and paediatric and adolescent childhood cancer survivors (paper III). Observational studies can only find associations between exposure and outcome and not evidence on causes and effects. The ability of the studies to achieve high internal and external validity was dependent on reliable measurement of the exposure and outcome variables and avoidance of systematic errors such as bias and confounders. In the following part of the thesis, this will be discussed.

A general strength of the studies described in paper I, II, and III was the study designs, as they were designed as prospective population-based cohort studies, and well-matched control groups, which reduced the risk of confounding.

The PEP study population was from the western region of Norway, and the PACCS study population was from the western and south-east parts of Norway. Free access to health care for all children and an egalitarian social structure in Norway make regional-specific health differences small, thus, regional study populations might be representative for the country in general. However, generalizability is likely to be limited to survivors of preterm birth and childhood cancer born in countries with similar health care strategies and treatment options.

12.2 Selection bias

Selection bias is present when the participants in a study are not representative of the population the study is intended to describe.

12.2.1 PEP

At the time of enrolment, there was low risk of selection bias in the PEP study, as all infants born in Western Norway with GA <28 weeks or birthweight <1000g were

included. Data on perinatal characteristics, intensive care unit stay, and pregnancy were available for all participants by data from the intensive care units and the Medical Birth Registry. Endothelial function (paper I) and myocardial function (paper II) were investigated in only 34% and 42% of the eligible PB/ELBW, respectively. Those who did not participate had similar GA, similar days of ventilatory support and oxygen demand, and higher (paper I) or similar (paper II) occurrence of moderate or severe bronchopulmonary dysplasia [150] compared to those who did participate. The non-participants had a slightly, but non-significantly, lower mean birthweight compared to the participants. A possible selection bias could therefore most likely have led to an underestimation of the differences in FMD and echocardiographic measured myocardial function between PB/ELBW and controls since lower birthweight is associated with higher cardiovascular risk.
There was low risk of selection bias of controls at the time of enrolment as they were identified using birth protocols from the maternity ward. Each PB/ELBW was matched with the next-born child with similar sex, GA >37 weeks, and birthweight >3000 grams. However, the participation rate of the controls was only 35% for assessment of endothelial function (paper I) and 46% for assessment of myocardial function (paper II), so selection bias may have occurred. If the controls with increased risk of cardiovascular disease were more prone to participate due to concerns about heart disease, this may have led to a selection of controls with the lowest FMD and myocardial function which would potentially reduce the difference in FMD and myocardial function between PB/ELBW and controls.

12.2.2 PACCS

The PACCS study protocol planned enrolment of 150 participants from work package 1 to further participate in work package 2. Only 62% of the eligible CCSs from work package 1 participated in the study presented in paper III, but none was excluded due to severe heart failure, activity triggered arrhythmia, or too low physical capacity. However, there is still a risk of selection bias since physically challenging testing was part of the study. This may have led to a selection of the fittest CCSs, which could introduce an overestimate of LV or RV function in the CCSs group. Our

study population of 128 CCSs was part of a larger PACCS sub-study population of 157 CCSs where characteristics of participants and non-participants have been compared [151]. Analyses of participants (n= 157) compared to non-participants (n= 89) reported similar age, sex distribution, age at diagnosis, time since diagnosis, and cancer diagnosis [151]. However, since participants in that sub-study also underwent cardiopulmonary exercise testing, one could still not rule out selection bias due to physically challenging testing.

Healthy, age- and sex matched schoolmates or friends were recruited as control participants by the CCSs. This may have led to selection bias as the CCSs may have recruited controls with same leisure interests and activity level as themselves. Consequently, an underestimation of differences in physical fitness and myocardial function between CCSs and controls may have occurred. Additionally, controls with personal interest in examination and testing due to health problems or perhaps health advantages they want to demonstrate may easier have accepted the invitation to participate in the study. If controls with health problems were overrepresented, this may have led to findings of a poorer myocardial function and peak VO_2 in the control group which could have led to reduction of the difference in results between CCSs and controls. Contrary, if the controls with health advantages were overrepresented, a greater difference in myocardial function and peak VO_2 between CCSs and controls could be expected. The control group was small with only 23 participants which reduces the validity of the study result. Additionally, the controls were recruited only from one of the two centres (Bergen).

12.3 Information bias

Information bias is present when one or several variables are incorrectly measured or classified and lead to an incorrect association between exposure and outcome.

12.3.1 Inclusion and exclusion PEP

The study was strengthened by well-defined inclusion criteria based on GA and birthweight. Neonates born at GA <29 weeks or with birthweight <1000 g were

included. GA was mainly (95%) determined by routine ultrasonography at 17–18 post-menstrual weeks which is reported to have an error of less than 7 days compared with GA obtained from *in vitro* fertilization [152]. If results from the ultrasound scan were unavailable, GA was determined using the date of the mother's last menstrual period which is considered a less reliable method.

Measurement of birthweight can be associated with a small measurement error depending on potential operator errors or scale used on the weight.

Measurement errors in determining birthweight and GA might have occurred, resulting in incorrect determination of whether the new-born was eligible for the study or not. Most likely these errors occurred infrequently with only a small risk of misclassification.

12.3.2 Inclusion and exclusion PACCS

Well-defined inclusion and exclusion criteria strengthened the study. The inclusion criteria were based on diagnosis (medically verified cancer), age (9-18 years), and time after finished treatment (>1 year ago). Since this was based on information from medical records, misclassification was unlikely. Selection was not biased by the exclusion criteria since none of the participants were excluded due to these criteria, and none remained included despite exhibiting these criteria.

12.4 Methodical considerations and reliability of outcomes

In general, several factors strengthened the reliability of the study results. Information regarding group affiliation (case or control) was unknown to the sonographer and the health personnel at the cardiopulmonary exercise test laboratory at the time of examination and data analysis. Imaging of the brachial artery and myocardium was performed by highly trained sonographers, with the same ultrasound system, and all offline analyses were made by one cardiologist with the same echocardiographic software. The calculated intra- and inter-rater variability of FMD and echocardiographic strain results and estimates of MW were good to excellent [153].

12.4.1 Endothelial function measured by FMD

To diminish the FMD-procedure related variance, the measurements were performed by a highly trained single sonographer with strict adherence to the International Brachial Artery Reactivity Task Force [93]. Measurements of endothelial function done by post-occlusive FMD of the brachial artery are well established [83, 154]. However, measurements of the vasomotor responses of epicardial coronary arteries to acetylcholine by coronary angiography are consider the golden standard of endothelial function assessment. The concordance between the coronary endothelial response quantified on the angiogram and the brachial endothelial responses measured by Doppler flow measurements, when both tests are performed simultaneously, is reported to be only modest [92]. Nevertheless, FMD is a preferable method to assess macrovascular endothelial function because it is non-invasive, requires equipment available at any department of cardiology, is painless, and has a short examination time (about 30 minutes).

12.4.2 Speckle-tracing echocardiography

Echocardiography continues to be the preferred method in evaluation of myocardial function. Traditionally, definitions of and guidelines regarding management of myocardial dysfunction have been based on measures of EF. Since EF (biplane Simpson) is derived from measures of LV area, it relies on geometric assumptions and is extremely load dependent. Thus, EF may change with altered geometry and loading conditions and does not necessarily reflect true myocardial contractility [155]. In later years, echocardiographic speckle-tracking derived strain analysis has provided incremental information on global and regional myocardial function and allowed detection of subclinical early stages of LV dysfunction [156], and its clinical relevance and role in risk stratification are increasing [144, 156]. Even though speckle tracking derived strain is reliant on 2-dimensional image quality, frame rate, heart rate, transducer transmitting frequency, and the accuracy of the determination of region of interest, the semi-automated nature of speckle-tracking echocardiography provides good intra-rater and inter-rater reproducibility. Additionally, this is a less angle-dependent method compared to measures of ejection fraction [116, 157]. Although less

load-dependent compared to measures of EF, measures of longitudinal strain are also influenced by loading conditions. Increasing afterload is associated with decreasing EF and LV-GLS [158, 159] and increasing PSS [160].
To exclude vendor related variability of the outcomes, the same ultrasound system, echocardiographic software, and stored data format (DICOM), were used during examination and analyses in all study participants.

To ensure correct measurements, all LV, RV, and LA strain analyses were done according to recommendations from the European and American societies of echocardiography [143, 144, 146], and images compromised by artefacts, or otherwise inappropriately tracked, were excluded. Compared to imaging of the LV, it can be challenging to obtain RV images with good enough signal quality of both the lateral wall and the apex to allow speckle-tracing. Additionally, tracking of the RV apex can be demanding due to its shape. Speckle-tracking of the LA is also known to be more challenging compared to the LV due to the thin walls, frequently extremely mobile atrial septum, and that the orifices of the pulmonary veins and LA appendage require an extrapolation of the LA contour [161]. As a result of this, less RV and LA than LV strain results were obtained. Measures of LV, RV, and LA strain in the control groups were close to previously described reference values for healthy populations within the same age range. This strengthens the reliability of the results.
While reference values and guidelines on LA strain are based on measures of two- and four chamber view images, only four chamber view images were used for analyses of LA strain in our study. However, we still found quite similar mean LA peak longitudinal strain in our control group (39.7%) compared to former reported reference values in healthy subjects (39%).

Measures of PSS, calculated by PSI in the different wall-segments, are subject to the same limitations as GLS measures in general [113]. However, the measures are probably more vulnerable to inaccuracy and increased intra-rater variability since they are regional measures [112]. Additionally, a reliable timing of the aorta valve closure is important to determine the presence or not of PSS, and amplitude of PSI.

To reduce variability in measures of aorta valve closure between study participants, the same modality (continuous-wave Doppler) was used for this measure in all participants. Analyses of intra-rater and inter-rater variability for the PSI results were not conducted, which represents a limitation to the study. Neither could we compare the PSI results in the control group to reference values to increase validity of the result, as such reference values are not yet established in paediatric populations. However, former studies of healthy and low-risk middle aged populations have reported mean and median global LV PSI values of 1.2% and 2.0%, respectively [107, 113]. These values are quite similar to those found in our paediatric control group (mean global LV PSI of 1.8%).

12.4.3 Myocardial work

All MW analyses were done by one trained cardiologist and as recommended by Russell et al. [118]. Non-invasive analyses of MW are based on establishment of opening and closure time of the mitral valve, measures of resting blood pressure at time of the echocardiographic examination, and measures of LV-GLS to derive a LV pressure-strain curve. Since work by definition equals length times force, the use of strain and pressure does not provide a direct measure of work, but can be used as a valid estimate of it as the method is found to strongly correlate to invasively measured MW [117, 118].

To reduce analysis dependent variability, the same modality for timing of the mitral valve opening and closure, pulse-waved Doppler, was used in all image analyses. Blood pressures were measured with an automated oscillometric device to reduce healthcare personnel dependent variability compared to manually performed measurements. Measurements and limitations of LV-GLS are described above. Analyses of MW have been considered an advancement of LV strain analyses due to reduction of the strain analysis` afterload dependent limitations [119, 120]. However, because it is derived from strain measurements, the method cannot be considered afterload independent. Additionally, the method is criticised for neither incorporating LV end-diastolic pressure (preload) which affects the overall pressure-strain loop area, nor incorporating afterload generated from arterial stiffness and vascular

resistance since the afterload parameter is obtained from brachial artery measurements and not the aorta [116]. Our study population had normal measures of diastolic function, including normal LV reservoir function which has been reported to detect increased LV filling pressure at an early stage. Thus, we could assume the LV filling pressure was low.

Although measures of MW have not formerly been investigated in preterm born young adults, we found quite similar means of GWI, GCW, GWW and GWE in the control group as formerly described reference values [120]. This, in addition to good-to excellent results for intra-rater and inter-rater variability of GWI, increases the reliability of the results.

12.4.4 Cardiopulmonary exercise test

A cardiopulmonary exercise test on a treadmill with breath-by-breath analyses of the individual`s expired gases provide direct measures of VO_2 during the test. This benefits the reliability of the peak VO_2 results compared to peak VO_2 measured by indirect methods. The test protocol was unambiguous, decreasing the risk of discrepancies in interpretation of the protocol between the centres. Additionally, the test was performed by experienced personnel, minimizing the risk of participants not being able to demonstrate their maximal exercise capacity. Most participants were able to cope with the test demands and completed technically satisfactory maximal exercise tests. Only data from 2 CCS and 1 control were missing.

Peak VO_2 is normally mainly limited by the ability of the cardiorespiratory system to deliver oxygen to the exercising muscles [125], but other factors, such as deconditioning, musculoskeletal and respiratory dysfunction, and lack of motivation may also influence the result. Since cancer treatment is associated with multiple adverse effects, these factors might have influenced on the peak VO_2 results. Thus, complex mechanisms could have contributed to the association between decreasing myocardial function and decreasing peak oxygen consumption in the CCSs.

12.5 Confounding and mediators

A confounding variable is an extraneous variable correlated with both the exposure and the outcome variable which affects the results when the relationship between the exposure and outcome is analysed. If a confounder is not adjusted for in the analysis, the result will be biased because the effect of the exposure is mixed with the effect of the confounder. A mediator is a variable which partly (or completely) explains the process through which the exposure and outcome are related.

Although the studies described in paper I, II, and III included well matched control groups which reduced the risk of confounding, some possible confounding variables were identified based on a clinical and empirical understanding of factors known to be associated with the exposure and outcome variables and confirmed by linear regression analyses. These variables were adjusted for during the analyses.

In paper I, analysis of the absolute difference in FMD between PB/ELBW and controls was adjusted for brachial artery baseline diameter as the PB/ELBW group had lower BSA than controls (1.8 kg/m^2 in PB/ELBW versus 1.9 kg/m^2 in controls, $p < 0.001$). Thus, the PB/ELBW group was more likely to have a smaller brachial artery than the controls (baseline diameter 3.21 mm in PB/ELBW versus 3.37 mm in control, $p = 0.17$), which could overestimate the FMD in the PB/ELBW group [162]. Although the PB/ELBW and control group were well matched for several known cardiovascular risk factors, a limitation to the study was that no information regarding family history of cardiovascular disease or cholesterol levels was available. Previous studies have not found an association between maternal familial hypercholesterolemia and low birthweight or preterm birth [163], but a possible association between maternal dyslipidaemia and preterm birth [164]. A small risk of unknown confounding could therefore not be ruled out. However, as all study participants had normal blood pressures, all measurements of HbA1c were well beneath the cut-off for prediabetes, and the BMI was similar in the PB/ELB and controls, there was probably not a high risk of lipid discrepancy between the groups. Additionally, the measurements of HbA1c were incomplete. However, the 13 study

participants with missing HbA1c had normal BMI (mean BMI 21.8 kg/m^2), indicating a low risk of metabolic syndrome in these subjects.

In paper II and III, analyses of the difference in LV and RV strain measurements between the PB/ELBW and controls, and between the CCSs and controls, were adjusted for BSA as the PB/ELBW group had lower BSA compared to controls and the CCSs had a tendency of higher BSA compared to the controls (1.5 m^2 in CCSs versus 1.4 m^2 in controls, p= 0.09) which could systematically bias the strain measurements results [147, 165]. Additionally, regression analyses revealed significant influence of echocardiographic transducer (5Sc (1.5-4.6 MHz) or 4Vc-D (1.4-5.2 MHz)) on the strain results. This was probably due to a higher risk of foreshortening with use of the 4Vc-D transducer. To address this issue, as well as the single centre inclusion of controls in paper III with consequent use of only one transducer in all controls, correction for transducer was added in the statistical analyses.
LV strain measurements are known to be afterload dependent in terms of more reduced LV-GLS with higher blood pressure [158]. This was not adjusted for when the strain results for PB/ELBW and controls or CCSs and controls were compared. This would most likely not influence the result in the PEP study, as the mean blood pressure in the PB/ELBW group was similar as the mean blood pressure in the control group (113/71 mmHg (SD 12/8) and 112/70 mmHg (SD 10/8), respectively). However, in the PACCS study, the mean systolic blood pressure was higher in the controls compared to the CCSs (112 mmHg versus 107 mmHg, respectively (Table 6)), which may have led to underestimation of the difference in systolic myocardial function between CCSs and controls.

In paper III, the correlation between peak VO_2 and LV-GLS was adjusted for sex as males are known to have a higher peak VO_2 than females [166] and the sex distribution between the groups was slightly unequal (52% males in the CCSs group versus 44% in the control group, p= 0.5). The correlation analysis was also adjusted for age, as the mean age was higher in the CCSs compared to the controls (13.6 years (SD 2.6) versus 12.7 years (SD 3.1), respectively, p= 0.04) and VO_2 expressed by ml/kg/min are reported to remain stable in boys while tend to decline in girls (20% decrease

between 8 to 13 years of age) [167]. Additionally, regression analyses revealed significant influence of location on peak VO_2 results in the CCSs examined in Oslo versus those examined in Bergen, which could not be explained by difference in chemotherapy doses, radiation therapy, blood pressure, or other characteristics. There might be technical or procedure related differences in exercise test results at the two centres. Due to this, and the single centre inclusion of controls, adjustment for location was added in the analyses.

12.6 Statistical power

An important limitation of the studies presented in this thesis was the relative small study population sizes. Power calculations were not performed for the research questions presented in papers I, II and III. It is possible that some results, especially sub-group analyses, were incorrectly reported as insignificant due to lack of statistical power because of small study sample sizes (type II error).

Paper I and II

The study presented in paper I and II is part of the PEP study which included the participants in year 2000, therefore, we could not influence on the study population size in our sub-study. Analyses of endothelial function by FMD and echocardiographic measured LV-GLS, in study populations of young adults born with similar degree of prematurity, have rarely been done before, and mainly with findings of normal FMD and LV-GLS. There is also lack of former studies of MW in preterm born young adults. Therefore, post hoc power analyses were based on detecting a difference between PB/ELBW and controls of 0.75 SD from reported normal values in healthy populations with similar age range. Post hoc power analysis (with alfa 0.05 and power (1- beta) 0.8) for detecting a 33% (equivalent to 0.75 SD) difference in FMD [168], a 10% (equivalent to 0.75 SD) difference in LV-GLS [159], and a 10% (equivalent to 0.75 SD) difference in GCW [169] between groups, generated a sample size of 29, 32, and 29 participants, respectively, suggesting adequate power for comparing the preterm born participants to the controls. However, the statistical power to detect smaller differences between subgroups was probably too low.

Paper III

During planning of the PACCS study, sample size calculations were based on an expected 10% difference in peak VO_2 between CCSs and controls [141]. With a statistical power of 80% a sample size of minimum 23 CCSs was estimated to detect such a difference. To facilitate subgroup analyses, 150 CCSs were included in the study. Post hoc power analysis (with alfa 0.05 and power (1- beta) 0.8) for detecting a 10% (equivalent to 0.75 SD) difference in LV-GLS [105, 170, 171] between groups generated a sample size of 29 suggesting adequate power for comparing the CCSs participants to controls. However, the statistical power to detect smaller differences between subgroups was probably insufficient.

12.7 Discussion of main results

12.7.1 Endothelial function in young adults born very preterm or with extremely low birthweight

We found reduced FMD and endothelial-dependent dilatation capacity of the brachial artery in 50 young adults with mean age 28 (SD 6) years born preterm at mean GA 27 (SD 2) weeks and with mean birthweight 961 (SD 225) g compared to term-born controls with similar cardiovascular risk profile. Lower birthweight and GA were associated with more reduced endothelial function.

Since reduced FMD indicates endothelial dysfunction, which constitutes an early step in the development of atherosclerotic cardiovascular disease, our finding adds to the increasing evidence of an association between preterm birth and elevated future cardiovascular risk. Only a few studies have investigated endothelial function in study populations similar to ours. Bassareo et al.[133] found reduced microvascular endothelial function measured by finger plethysmography in young adults with mean GA 27.8 weeks (SD 2.2) and mean birthweight 838 g (SD 116), while Flahault et al. [132] reported normal endothelial function measured by FMD of the brachial artery in young adults with mean GA 27.2 weeks (SD 1.4) and mean birthweight 963 g (SD 225). Two other studies by Shingal et al. [130] and Hovi et al. [131] also reported normal endothelial function measured by FMD in adolescents and young adults, but these study participants were born with higher GA (mean GA 31 and 30 weeks,

respectively) and with higher birthweight (both mean 1400 g) compared to our study population, which could explain the difference in results since birthweight and GA have been inversely related to increased cardiovascular risk [5, 6].

Reduced FMD reflects endothelial dysfunction, and reduced NID might reflect vascular smooth muscle cell dysfunction and vascular structure alternations due to established atherosclerosis [95-97]. Both would result in reduced ability of vascular dilatation. Although the interrelationship between FMD and NID is debated [95, 98-100], a low FMD/NID ratio could indicate cardiovascular risk factors impairing the endothelial function, but no established vascular disease, while subjects with established vascular disease and vascular smooth cell dysfunction might have normal or even high FMD/NID ratio despite endothelial dysfunction. Describing endothelial function as endothelial-dependent capacity is probably most valuable when NID is normal and can be estimated as maximal dilatation. Since our study population was young with mean age 28 years, and none had multiple cardiovascular risk factors or established atherosclerotic disease, we expected to find normal NID in most participants, which was confirmed. Only 8% in the PB/ELBW group and 14% in the control group had NID <15.6% which has been suggested as an age-independent cut-off value [168]. The participants with NID <15.6% were non-smokers and all had normal values for HbA1c and resting blood pressure. Only one PB/ELBW and three controls were overweight with BMI >25 kg/m^2. Based on this, our findings of decreased FMD and endothelial-dependent dilatation capacity in the PB/ELBW compared to controls could indicate impaired endothelial function, but probably preserved vascular smooth cell function. Thus, advanced stages in the atherosclerotic process were likely not reached.

Cardiovascular risk factors such as tobacco use, hypertension, obesity, hyperlipidaemia, insulin resistance, physical inactivity, and increasing age are associated with endothelial dysfunction [79, 86]. Since the PB/ELBW and control group had low and similar cardiovascular risk profile (age, smoking, BMI, blood pressure, diabetes mellitus), extremely low birthweight and very preterm birth might represent an individual risk factors for endothelial dysfunction. This finding provides valuable

information on the influence of GA and birthweight on vascular function in young adulthood, and further, the lifetime risk of atherosclerotic disease.

Similar to prior studies [5, 6] we found an inverse association between birthweight and GA and cardiovascular risk, as lower endothelial function was associated with lower birthweight and GA. The lower mean birthweight in the youngest PB/ELBW cohort compared to the oldest cohort (885 g versus 1022 g) could have led to the finding of lower mean FMD in the youngest cohort. However, we were not able to show a significant difference in endothelial function between the PB/ELBW cohorts. This was probably due to too small study samples within the cohorts of the PB/ELBWs to reveal minor group differences. However, the possible improvement in neonatal intensive care treatment and the younger age at the time of assessment could have favourable influence on FMD in the younger cohort and explain the result.

12.7.2 Myocardial function in young adults born very preterm or with extremely low birthweight

63 young adults born preterm at mean GA 27 weeks (SD 2) and with mean birthweight 945 g (SD 217) were compared to term-born age- and sex matched controls with similar cardiovascular risk profile. The PB/ELBW had reduced LV-GLS compared to term-born controls, and lower birthweight was associated with more reduced LV-GLS. Estimates of MW and diastolic function, including measures of LA longitudinal strain, were similar in PB/ELBW compared to controls, and increasing chronological age was associated with decreasing diastolic function.

In agreement with our findings, former studies of myocardial function of young adults born preterm or with low birthweight have mainly reported preserved EF, but reduced LV systolic function measured by strain [134-136]. This corresponds well with the described superiority of strain compared to EF in order to reveal early stages of LV impairment. Our finding of reduced LV-GLS in the PB/ELBW group compared to controls is suggestive of increased risk of developing heart failure in the preterm born participants. Additionally, lower birthweight was associated with lower LV-

GLS, suggestive of poorer prognosis with lower birthweight. This is in agreement with former epidemiological studies which have reported increased risk of heart failure in individuals born preterm or with low birthweight [23, 26], and an inverse association between GA and birthweight and the risk of cardiovascular disease [5, 6]. However, results from former clinical studies have not been consistent, and echocardiographic measured LV strain in preterm born young adults quite similar to our study participants has also been reported normal [172].

Even though former studies have reported reduced diastolic function in preterm born young adults [137], we found normal and similar diastolic function in our preterm born study participants compared to the controls. This was supported by the finding of normal measures of LA reservoir strain which have been reported to detect increased LV filling pressure at an earlier point than more conventional echocardiographic diastolic parameters like LA volumes and mitral inflow and annular tissue velocities [122, 123]. Aging is associated with reduced LV relaxation, which may lead to diastolic dysfunction [145]. This also applies for young adult populations [173]. This corresponds well with our finding of an association between increased chronological age and lower diastolic function.
Cardiac remodelling and myocardial fibrosis, with consequently morphological LV alterations, are found in most studies investigating preterm born adults [136]. Diverging results regarding systolic and diastolic myocardial function between studies may reflect the complex interplay of altered cardiac structure and loading conditions on measures of systolic and diastolic function in early stages of myocardial dysfunction in young study populations.

EF and LV longitudinal strain are afterload dependent measures of myocardial function, with decreasing EF and longitudinal strain with higher blood pressure [158]. Since analyses of MW incorporate afterload, in terms of blood pressure measurements, analyses of MW have been considered an advancement of LV strain analyses [119, 120]. Combined, measures of LV-GLS and estimates of MW might be used to differentiate between reduced contraction patterns caused by increased myocardial

workload or actual attenuated contractility. This may be especially important in evaluation of myocardial function in preterm born adults, who have increased prevalence of hypertension [174].

Newly emerged hypertension will result in findings of reduced EF and LV-GLS, but increased GWI (total work) and GCW (work which contributes to ejection)[158], while reduced myocardial contractility will result in impairment of all these parameters, and additionally, a parallel increase of GWW (work which does not contribute to ejection). The additive value of these new parameters describing myocardial function is still object of study, and MW has not formerly been evaluated in preterm born young adults. MW is influenced not only by the contraction power of the myocardial fibres, but also by the wall stress applied on the LV segments and the LV loading conditions [116]. Since the PB/ELBW and controls in our study had similar means of parameters describing LV filling pressure, heart rate, blood pressure (112/69 mmHg in PB/ELBW and 111/69 mmHg in controls), LV end-diastole internal diameter, and myocardial mass index, we could assume that the two groups had similar loading conditions as well as wall stress applied on the LV. We found a tendency towards reduced GWI and GCW, and higher GWW, in the PB/ELBW group compared to the controls, although not significantly different. This is in line with our findings of mainly normal myocardial function in the PB/ELBW group, however, suggestive of presence of cardiac remodelling and elements of myocardial fibrosis [136].

The protocol of the MW method incorporates measures of brachial artery blood pressure which equals the LV pressure if LV outflow gradients are absent, but does not account for afterload generated by arterial stiff and vascular resistance [116]. Since premature birth is associated with increased arterial stiffness [175, 176], this could result in an increased myocardial workload in the PB/ELBW group compared to the controls, and consequently findings of higher measures of GWI and GCW. This could explain why LV-GLS was significantly reduced in the PB/ELBW group compared to controls, but not measures of GWI and GCW despite similar blood pressure in the two groups.

12.7.3 Sex differences in endothelial function and myocardial function in PB/ELBW

Although argued to be due to calculation bias based on brachial artery baseline diameter differences between sexes [162, 177], a sex-related difference in FMD has been described [79]. Additionally, both sexes show an age-dependent decline in endothelial function [79], and a steeper decline is seen after menopause in women [87]. However, we found no sex differences when the absolute FMD diameter change adjusted for baseline diameter was compared between PB/ELBW and controls.

Although all-cause mortality and morbidity of preterm born young adults are reported higher in men compared with women [5], the difference in LV-GLS between PB/ELBW and controls in our study was not significantly greater in the male versus female participants. Both results can be due to small sample sizes and too little statistical power for the interaction analyses.

12.7.4 Myocardial function in paediatric childhood cancer survivors

128 paediatric CCSs with mean age 13.6 (SD 2.6) years were compared with 23 age- and sex matched healthy controls. The LV, but not RV, systolic function was significantly reduced in the paediatric CCSs compared to the controls, however, mainly within normal range. Higher treatment doses of anthracyclines and increasing time after cancer treatment were associated with lower LV and RV systolic function, and lower LV myocardial function was associated with lower peak VO_2.

Former studies have described increased cardiovascular risk in survivors of childhood cancer [7, 45, 178, 179], and although not so thoroughly studied, this seems to be detectable already in childhood. Some previous studies have reported reduced LV myocardial function measured by strain in anthracycline treated young CCSs [105, 171, 180]. This corresponds well with our finding of reduced LV-GLS in paediatric CCSs compared to controls. However, most of our CCSs had systolic function within normal range, only 13% had LV-longitudinal strain z-score < -2. This finding is similar to the study by Slieker et al.[171] which reported impaired LV longitudinal strain in 8% of 510 paediatric CCSs treated with similar mean anthracycline dose (150 mg/m^2) as our

study participants. Despite the finding of mainly normal myocardial systolic function in our CCSs, we found a higher share of wall-segments with any degree of PSS in the CCSs compared to the controls. Presence of PSS has to our knowledge not previously been investigated in CCS, and the mechanisms behind the finding are uncertain, although might be due to myocardial micro-infarction related to the cancer treatment and/or the cancer disease. Since PPS has been reported to be a predictor of cardiovascular morbidity and death [113, 114], our finding indicates that PSS might add as a marker of increased cardiovascular risk in the CCSs.

As previously reported [50], we found an inverse association between myocardial function and anthracycline dose. But noticeable, LV longitudinal strain z-score was found reduced also in the subgroup treated with low doses of anthracyclines and in the anthracycline naive CCS participants, compared to controls. This finding suggests that no safe dose of anthracycline exists and supports former reports of associations between vinca alkaloids or platinum derivates and heart failure [56, 57]. RV-LS was mainly normal and not significantly different in CCSs and controls. However, since progressive cardiomyopathy after cancer treatment has been reported, and we found an association between increasing time after cancer treatment and more reduced both LV-GLS and RV-LS, we could expect development of RV dysfunction with time. This is consistent with previous findings of reduced RV function in adult CCSs, but not in paediatric CCSs [51, 52, 171].

Myocardial dysfunction, exercise intolerance, and reduced peak VO_2, are described as prevalent in adult CCSs [62]. Because these characteristics are associated with increased mortality [62], they are important to identify also in young CCSs. Cardiopulmonary exercise testing might be useful to identify CCSs with subclinical myocardial impairment [63] and reduced myocardial reserve. However, only a few former studies have investigated the relationship between myocardial function and peak VO_2 in paediatric CCSs, and the results have been diverging. Both reduced [66] and preserved [181] peak VO_2 have been reported in paediatric CCSs with normal myocardial function measured by fractional shortening, compared to controls. Others have reported

reduced peak VO_2 in paediatric CCSs with reduced myocardial function revealed by cardiac imaging during exercise [65, 182], but also in CCSs with preserved myocardial stress response [65].

We found that lower LV myocardial function measured by LV strain in the CCS was associated with lower peak VO_2. Although more complex mechanisms may also have contributed, the result was likely due to decreased cardiac output [126] and myocardial reserve, implying clinically significant reduction of left ventricular function.

12.7.5 Sex differences in myocardial function in CCSs

Female sex has been associated with increased treatment-related cardiovascular morbidity in CCSs [7], but we found no such association. This may be due to small sample sizes (particularly the small control group) and insufficient statistical power for the interaction analyses. Another explanation can be the moderate mean dose of anthracyclines (152 mg/m^2) used in our study compared to other studies. Kramer et al. [183] summarized ten studies from the late 90s to the early 2000s in which the CCSs had been treated with mean anthracycline doses mainly above 250 mg/m^2. Of these, four studies reported an association between female sex and decreased myocardial function.

13. Relevance and clinical implications of study findings

13.1 PEP

The findings of reduced FMD and LV-GLS in the PB/ELBW compared to controls are suggestive of elevated risk of developing atherosclerotic cardiovascular disease and heart failure in this population, already at young adult age, and lower birthweight was associated with worse outcome. Large cohorts of extremely and very preterm born are now entering adult life. For the time being, no routine cardiovascular follow-up or preventive strategies are established beyond the usual recommendations for the general adult population. It is of public health interest to reduce cardiovascular morbidity and need for extensive health resources in this growing group of adults. Follow-up programs with special attention to reduce modifiable risk factors for cardiovascular disease should therefore be considered. This could include age adjusted information on beneficial lifestyle already from preschool age, including dietary advice and encouragement to maintain adequate level of physical activity. Additionally, regularly follow-up in the primary healthcare service to detect onset of treatable conditions such as hypertension and dyslipidaemia could be recommended from adolescent age, and regularly follow-up by cardiologist from young adult age.

13.2 PACCS

We found reduced LV systolic myocardial function in paediatric CCSs compared to healthy controls, indicating a detectable increased risk of developing heart failure in CCSs already in childhood. The LV myocardial function was found reduced also in CCSs treated with low doses of anthracyclines and increasing time after treatment was associated with lower myocardial function. The present follow-up guidelines on cardio-oncology [74] are based on cardiovascular risk stratifications of the patients after finished treatment. These guidelines recommend to consider echocardiographic examination every 2 years in high risk CCSs and every 5 years in moderate risk CCSs, while no echocardiographic follow-up is recommended in CCSs treated with anthracycline doses <100 mg/m^2 [74].

Our findings imply that long-term follow-up, including monitoring of myocardial function by echocardiographic examination and perhaps also by exercise test, might

be indicated for all CCSs, regardless of cumulative anthracycline treatment dose. Education of adolescent and adult CCSs and their health care providers to ensure attention to the increased cardiovascular risk, and regularly screening for modifiable cardiovascular risk factors, are already implemented in the cardio-oncology follow-up recommendations. Regular physical activity, including high intensity exercise, is recommended in CCSs with normal LV function or with asymptomatic myocardial dysfunction [73]. Although not much studied in CCSs, exercise is suggested to prevent development of cancer treatment-related myocardial dysfunction in paediatric and adolescent cancer survivors [70], thus, physical activity might be especially important in CCS without clinical signs of heart failure and should therefore be encouraged.
The many survivors of childhood cancer have an increased lifetime risk of developing cardiotoxic treatment-induced myocardial dysfunction. It is of public health interest to generate prevention strategies to reduce cardiovascular morbidity in this group already from young age. And also for this group, lifestyle advice should be introduced already in preschool age to encourage lifelong beneficial lifestyle habits.

14. Conclusions

The overall conclusion of the studies presented in this thesis was that Norwegian young adults born very preterm or with extremely low birthweight and paediatric childhood cancer survivors had increased risk of cardiovascular disease compared to sex- and age matched controls. The study conclusions can be summarised as follows by answering the research questions:

Answer to research question I: Young adults born very preterm or with extremely low birthweight born in Norway in the period from 1980 to 2000 had reduced flow-mediated endothelial-dependent dilatation and endothelial-dependent dilatation capacity of the brachial artery compared to controls, suggestive of reduced endothelial function which is established as an early marker of atherosclerotic vascular disease. Nitroglycerine-induced dilatation was preserved, indicating that early but not advanced stages in the atherosclerotic process were reached.

Answer to research question II: Young adults born very preterm or with extremely low birthweight born in Norway in the period from 1980 to 2000 had reduced global left ventricular function measured by longitudinal strain compared to controls, indicating increased risk of developing heart failure. Measures of diastolic left ventricular function, including measures of left atrial strain, and estimates of myocardial work were similar in the preterm born compared to the controls.

Answer to research question III: Paediatric and adolescent childhood cancer survivors treated in Norway had reduced left ventricular function compared to controls, suggestive of increased risk of developing heart failure. Higher treatment doses of anthracyclines and increasing time after cancer treatment were associated with lower myocardial systolic function, and lower left ventricular myocardial function was associated with lower peak oxygen consumption.

15. Future perspectives

The findings in this study of young adults born preterm and paediatric cancer survivors add information on their cardiovascular risk. Future health outcomes in preterm born and CCSs should be further explored, preferably by longitudinal follow-up studies and intervention studies, to identify those who would benefit most from closer follow-up, and to improve outcomes. This would gain the subjects at superior risk and be health-economically beneficial.

Moreover, since sex-related differences regarding cardiovascular disease have been shown to be significant, more studies should aim to investigate potential differences in outcomes related to sex to ensure adequate follow-up and treatment. It should also be investigated if a more aggressive approach to reduce modifiable cardiovascular risk factors such as hypertension, dyslipidaemia, and obesity by introducing medical treatment early could alter outcomes, or if introduction of conventional heart failure medication already in subclinical stages of cardiac dysfunction could improve the prognosis. Additionally, exploring if increased amount of physical activity already in childhood could reduce cardiovascular complications, or delay the onset of these, would be of great interest. A PACCS sub-study is already planned on this subject and will evaluate the feasibility of a 6-month personalized physical activity intervention as a preparation for a future well-designed large randomized controlled trial. A similar physical activity intervention study including the PEP study population, with special attention to cardiac function measured by echocardiography, would add valuable information on cardiovascular health in preterm born. Such a study could be facilitated at the Energy Center for Children and Adolescents at Haukeland University Hospital.

Furthermore, in an era with increased focus on mitigating the cardiotoxic effects of cancer treatment, studies of myocardial function in CCSs are of importance for future generations. Minimizing of treatment doses, facilitation of presumable favorable delivery of chemotherapy and irradiation, and the use of cardio-protectant medication such as the iron-chelating Dexrazoxane which inhibits the formation of doxorubicin-

iron complexes resulting in reduced generation of reactive oxygen species, must be done with caution to avoid reduced antineoplastic effects. Significant reduction of cardiotoxicity due to these treatment strategies should continuously be verified.

16. References

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Vascular Endothelial Function Assessed by Flow-Mediated Vasodilatation in Young Adults Born Very Preterm or With Extremely Low Birthweight: A Regional Cohort Study

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Background: Preterm birth and low birthweight have been associated with increased risk of cardiovascular disease in young adults. Endothelial dysfunction is established as an early marker for development of atherosclerotic cardiovascular disease. Previous studies of endothelial function in young adults born very preterm or with extremely low birthweight have, however, shown diverging results.

Objective: We aimed to evaluate the risk of cardiovascular disease as measured by vascular endothelial function in young adults born very preterm (<29 weeks of gestation) or with extremely low birthweight (<1,000 g), compared with term-born controls.

Methods: This study included 50 young adults born very preterm or with extremely low birthweight and 49 term-born controls born in Norway in the periods 1982–1985, 1991–1992, and 1999–2000 at mean age 28 (±6) years. The endothelial function was assessed by ultrasound measured flow-mediated dilatation (FMD) of the right brachial artery. The arterial diameter was measured at baseline, after release of 5 min of occlusion, and after sublingual administration of nitroglycerine. FMD was reported as absolute and percentage diameter change from baseline and relative to nitroglycerine-induced dilatation.

Results: The participants were mainly normal weight non-smokers, without hypertension, diabetes, or established cardiovascular disease. The cases and controls had mean blood pressure 112/71 (SD 12/9) and 112/69 (SD 11/8) mmHg, body mass index 24.0 (SD 4.2) and 24.4 (SD 4.5) kg/m^2, and HbA1c 32.7 (SD 2.5) and 33.0 (SD 2.6) mmol/mol, respectively. For both groups, 4 (8%) were smokers. Mean FMD for the adults born very preterm or with extremely low birthweight was 0.17 mm (95% CI 0.14, 0.21) vs. 0.24 mm (95% CI 0.20, 0.28) for the controls ($p = 0.01$), corresponding to a percentage increase of 5.4% (95% CI 4.2, 6.6) and 7.6% (95% CI 6.2, 8.9), respectively ($p = 0.02$). The FMD relative to maximal nitroglycerine-induced dilatation was 20% and 31%, respectively ($p = 0.001$).

Conclusions: Young adults born very preterm or with extremely low birthweight have significantly lower FMD compared with the term-born controls suggesting an increased risk of cardiovascular disease.

Keywords: endothelial function, flow-mediated dilatation, very preterm, extremely low birthweight, cardiovascular risk

INTRODUCTION

Preterm birth is an important cause of neonatal morbidity and mortality worldwide. In the 1970s, most neonates born extremely preterm (<28 weeks of gestation) in Norway did not survive. Technical and medical development in neonatal intensive care over the last decades have markedly increased survival rates for these neonates, which today exceed 80% (1–3). Neonates born extremely preterm now represent ∼0.5% of all children growing up in Norway (4).

There is increasing evidence indicating that children born preterm (before 37 weeks of gestation) or with low birthweight (LBW) (<2,500 g) carry a risk of poor long-term health outcomes and a higher risk of young adult death, even in individuals born only moderately to late preterm (2, 5) (see **Supplementary Table 1** for definitions of different degrees of preterm birth and low birthweight). Preterm birth and LBW have been linked to increased risk of hypertension (6, 7), diabetes (8, 9), and metabolic disease (9, 10), and it has been suggested that both preterm birth and LBW might constitute risk factors for later development of cardiovascular disease (5, 10–17). A recent meta-analysis concluded that LBW is associated with an overall increased risk of cardiovascular disease, coronary heart disease, and stroke (18). Furthermore, a population-based cohort study from the Nordic countries reported a 2-fold higher cardiovascular mortality in young adults born preterm in year 1967 to 2002 (2). The increased risk of all-cause mortality and cardiovascular disease are found to be inversely associated with gestational age (GA) and birthweight (2, 5).

A healthy endothelium is important for vascular homeostasis, and an appropriate balance of mediating factors regulates endothelial vasoconstriction and vasodilatation. A hallmark of healthy endothelial function is the nitric oxide synthesis and release by the endothelial inner lining of blood vessels in response to vasodilating stimuli (19). Vascular endothelial dysfunction constitutes an early step in the development of atherosclerosis and plays a role in the onset and progression of cardiovascular disease (19–22). Factors that adversely affect the endothelium include common cardiovascular risk factors such as age, hypertension, tobacco use, obesity, hyperlipidemia, insulin resistance, and physical inactivity (23). Endothelial dysfunction is also seen in patients with a family history of early cardiovascular disease but no other risk factors (23).

Previous studies of endothelial function in children and young adults born preterm or with LBW have presented diverging results, with findings of normal as well as reduced endothelial function (17, 24–28). Only a few studies have included young adults born extremely preterm or with extremely low birthweight (ELBW) (<1,000 g). However, Bassareo et al. (29) reported impaired microvascular endothelial function in young adults born with ELBW.

In the present study, we aimed to investigate how very preterm birth or ELBW may influence cardiovascular risk, by comparing the vascular endothelial function in young adults born at GA <29 weeks or with birthweight <1,000 g, with term-born controls.

MATERIALS AND METHODS

Study Design and Participants

The Project Extreme Prematurity, organized by the WestPaed Research group in Bergen, Norway, have included three regional cohorts of young adults born very preterm at GA <29 weeks or with ELBW <1,000 g, from now on referred to as PB/ELBW. The three cohorts also included individually age- and sex-matched term-born controls, from now on referred to as controls. The participants have been followed longitudinally since year 2000, and have previously been examined in 2001 and 2008. The cohorts included a total number of 148 PB/ELBW and 138 controls. Subjects born in 1982–1985 (cohort 1) and in 1991–1992 (cohort 2) were retrospectively recruited, while those born in 1999–2000 (cohort 3) were prospectively recruited. The inclusion and follow-up of these cohorts have been described previously (30, 31).

During the period, November 2017 to February 2020 individuals from the three regional cohorts were invited to a third follow-up, including a first-time non-invasive ultrasound-based examination of endothelial function. A total of 99 participants were examined, including 50 PB/ELBW (48% men) and 49 controls (41% men). Of the participants, 42 belonged to cohort 1, 29 to cohort 2, and 28 to cohort 3. HbA1c (glycosylated hemoglobin) was chosen as screening for diabetes. HbA1c is highly correlated with fasting blood glucose and established as a screening diagnostic test with cut-off for prediabetes 39 mmol/mol (32, 33). The analyses were performed in 39 (78%) of the PB/ELBW and in 47 (96%) of the controls. All participants went through a clinical examination including measurements of height, weight, and blood pressure. A flow diagram of the project participants is shown in **Figure 1**.

The Regional Committee for Medical and Health Research Ethics of the Western Norway Health Authority approved the study (REC 2017/0068). Written informed consent was obtained from all participants.

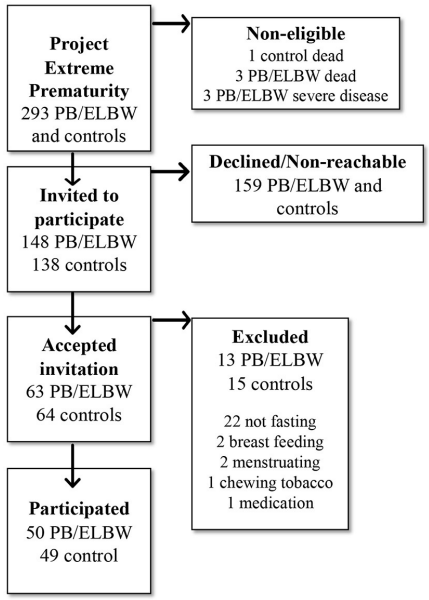

FIGURE 1 | Flow chart of the study population in the study of vascular endothelial function in young adults born very preterm or with extremely low birthweight (PB/ELBW) and term-born controls, part of the Project Extreme Prematurity in Norway.

Assessment of Endothelial Function by Flow-Mediated Dilatation of the Brachial Artery

Measurements of endothelial function by post-occlusive flow mediated dilatation (FMD) were performed according to guidelines by the International Brachial Artery Reactivity Task Force (34). Numerous factors affect flow-mediated vascular reactivity including the environmental temperature, ongoing infection, intake of food or drugs, and sex hormonal and sympathetic stimuli, among others (34). The assessment was therefore performed in supine position in a quiet, temperature-controlled room in the morning hours (before noon). Participant pre-test preparations included dietary restrictions (fasting for at least 8 h, and abstaining from caffeine and vitamin supplements on the examination day). In addition, all vasoactive medications and tobacco were withheld on the examination day, and the participants did not exercise the last 24 h before the examination. Participants who were pregnant, breast feeding, or menstruating were excluded to limit the variations in sex hormone levels in the female participants. Hormonal contraceptives have been shown to have little influence on FMD in healthy premenopausal women (35, 36), and participants using these were included in the study.

For imaging, we used Vivid E9 scanner (GE Vingmed Ultrasound, Horten, Norway) with a multiple linear array transducer (6 to 13 MHz). Offline imaging analyses were done with EchoPAC version 203 (GE Vingmed Ultrasound).

The participants were examined in supine position with the right arm resting in unchanged position. The right brachial artery was imaged above the antecubital fossa in the longitudinal plane, and the internal diameter was measured at end-diastole, from the anterior to the posterior endothelial lining of the blood vessel lumen. During image acquisition, anatomic landmarks such as veins, artery branches, and fascial planes were noted, and markers on the skin were drawn to help maintain the exact same image of the artery throughout the study. Occlusion of the brachial artery was done with a blood pressure cuff on the right forearm, inflated at least 50 mmHg above systolic blood pressure.

Images of the brachial artery were first collected at baseline after 10 min of rest, then repeatedly for 3 min after release of 5-min occlusion, and finally, after a second 10-min resting period, repeatedly for 5 min after a single dose (0.4 mg) of nitroglycerine spray (Nitrolingual; G.Pohl-Boskamp GmbH & Co.KG, Hohenlockstedt, Germany) was administered sublingually. Measurements were done at baseline (Dbaseline), at peak dilatation after release of occlusion (Dflowmediated), and at peak dilatation after sublingual nitroglycerine (Dpeaknitro). For all diameter measurements, an average of three was used. Further details and illustrations of the method are described in the International Brachial Artery Reactivity Task Force (34) and in **Figure 2**.

FMD was calculated as the absolute diameter change (Dflowmediated – Dbaseline) and the percentage diameter change {[(Dflowmediated – Dbaseline) / Dbaseline] × 100}. The endothelial-independent nitroglycerine-induced dilatation (NID) was calculated as the absolute diameter change (Dpeaknitro – Dbaseline) and the percentage diameter change {[(Dpeaknitro – Dbaseline) / Dbaseline] × 100}. The FMD relative to NID was measured to evaluate the endothelial-dependent dilatation capacity compared with maximal capacity [(Dflowmediated – Dbaseline) / (Dpeaktnitro – Dbaseline) × 100].

A single highly trained sonographer (B.E.) performed all FMD examinations. Another highly trained physician (E.L.) performed control measurements on images of the brachial artery in a random selection of study participant ($n = 12$). The interrater reliability as measured by a two-way mixed-effects model for absolute agreement was 0.99 (95% CI 0.96, 1.00) for Dbaseline measurements, 0.97 (95% CI 0.89, 0.99) for Dflowmediated measurements, and 0.94 (95% CI 0.82, 0.98) for Dpeaknitro measurements.

Information regarding group affiliation (PB/ELBW or control) was unknown to the sonographer at the time of examination.

Blood Pressure and Blood Sample Measurements

Blood pressure was calculated as the average of three measurements with an automated oscillometric device (Biolight BLT V6; Biolight Meditech Company, Guangdong, China) after 5 min of rest in supine position. Venous blood samples

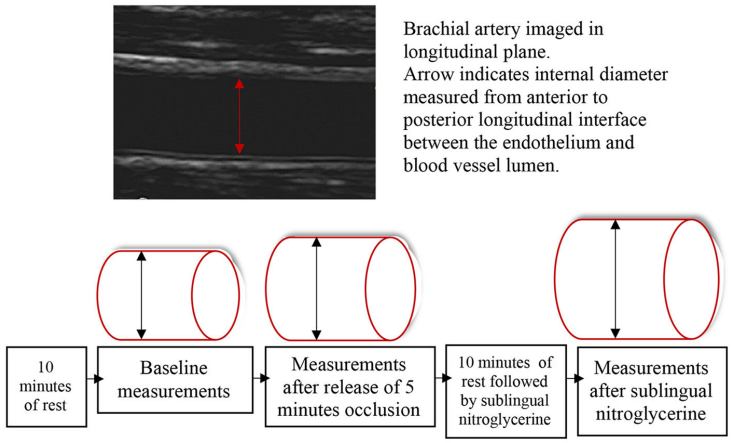

FIGURE 2 | The artery diameter was first measured at baseline after 10 min of rest. Thereafter, occlusion of the right brachial artery was done with a blood pressure cuff on the forearm, inflated at least 50 mmHg above systolic blood pressure for 5 min. The next measurements were done at peak artery dilatation after release of occlusion. After a second 10-min resting period followed by sublingual nitroglycerine administration, the last measurements were done at peak artery dilatation.

(in ethylenediaminetetraacetic acid–containing tubes) were collected for measurements of HbA1c.

Statistical Analysis

Gestational age is presented as medians with interquartile ranges (IQRs). Other descriptive variables and outcome data are presented as means with 95% CIs or SD. For comparison of the absolute flow-mediated and nitroglycerine-induced artery diameter changes in PB/ELBW and controls, analysis of covariance (ANCOVA) was used with adjustment for baseline diameter. To examine whether sex, age, BMI, HbA1c level, smoking status, or blood pressure could explain the difference in FMD between PB/ELBW and controls, these variables were also added to the regression model, and an omnibus test on an effect of at least one variable was performed. To examine whether the difference in absolute FMD between PB/ELBW and controls differed by sex, an interaction term for sex and group affiliation was added. For comparison of descriptive variables, percentage flow-mediated and nitroglycerine-induced artery diameter changes from baseline, and endothelial-dependent dilatation capacity (FMD relative to NID) between PB/ELBW and controls, independent samples *t*-tests (with equal variance not assumed) were applied.

Comparison of absolute and percentage FMD between subgroups of PB/ELBW was done with ANCOVA (with adjustment for baseline diameter) and independent *t*-test (with equal variance not assumed), respectively. Comparison of FMD between the three PB/ELBW cohorts was done by Welch's ANOVA. All tests were two-sided, and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

RESULTS

The characteristics of the study participants are shown in **Table 1**. The control group was significantly taller than the PB/ELBW group; otherwise, there were no significant differences for age, weight, body mass index (BMI), blood pressure, HbA1c level, or smoking status. In the PB/ELBW and control group, mean BMI was 24.0 (SD 4.2) and 24.4 (SD 4.5) kg/m^2, mean blood pressure 112/71 (SD 12/9) and 112/69 (SD 11/8) mmHg, and mean HbA1c 32.7 (SD 2.5) and 33.0 (SD 2.6) mmol/mol, respectively. For both groups, mean age was 28 (SD 6) years and 4 (8%) were smokers. The birthweight in the PB/ELBW group ranged from 550 to 1,480 g with mean birthweight 961 (SD 225) g. The GA ranged from 23 to 34 weeks with median GA 27 (IQR 2) weeks. Among the PB/ELBW participants, 14 (28%) were born small for gestational age [<10th percentile according to (37)], 28 (56%) were extremely preterm born (<28 weeks of gestation), 27 (54%) had ELBW (<1,000 g), 19 (38%) had birthweight ≤800 g or GA <26 weeks, and 11 (22%) had birthweight ≤700 g or GA <25 weeks. Mean baseline diameter of the brachial artery was not significantly different in the control group compared with the PB/ELBW group, 3.37 mm (95% CI 3.19, 3.54) vs. 3.21 mm (95% CI 3.07, 3.35), respectively ($p = 0.17$). None of the participants in the study had history of atherosclerotic cardiovascular disease.

Endothelial-Dependent Flow-Mediated Dilatation (FMD)

Table 2 describes measurements of FMD of the brachial artery. The mean absolute FMD was 0.17 mm (95% CI 0.14, 0.21) in the PB/ELBW group vs. 0.24 mm (95% CI 0.20, 0.28) in the control group ($p = 0.01$), and the mean percentage change was 5.4% (95%

TABLE 1 | Characteristics of study participants.

	PB/ELBW	Controls	*P*-value
Total, *n* (% males)	50 (48%)	49 (41%)	
Cohort 1 (born 1982–1985), *n*	21	21	
Cohort 2 (born 1991–1992), *n*	16	13	
Cohort 3 (born 1999–2000), *n*	13	15	
Gestational age (weeks), median (IQR)	27 (2)	Term-born	
Birthweight (g), mean (SD)	961 (225)	3,513 (304)	
Small for gestational age, *n*	14 (28%)		
Extremely preterm GA <28 weeks, *n*	28 (56%)		
Extremely low birthweight <1,000 g, *n*	27 (54%)		
Age (years), mean (SD)	28 (6)	28 (6)	0.87
Height (cm), mean (SD)	168.5 (8.2)	172.9 (7.7)	0.01
Weight (kg), mean (SD)	68.4 (14.8)	73.1 (13.5)	0.11
Smokers, n	4 (8%)	4 (8%)	0.98
BMI (kg/m^2), mean (SD)	24.0 (4.2)	24.4 (4.5)	0.59
HbA1c (mmol/mol) (SD)[a]	32.7 (2.5)	33.0 (2.6)	0.62
Systolic BP (mmHg), mean (SD)	112 (12)	112 (11)	0.86
Diastolic BP (mmHg), mean (SD)	71 (9)	69 (8)	0.38
Baseline diameter[b] (mm), mean (95% CI)	3.21 (3.07, 3.35)	3.37 (3.19, 3.54)	0.17

PB/ELBW very preterm born (<29 weeks of gestation) or with extremely low birthweight (<1,000 g). IQR, interquartile range; GA, gestational age; BMI, body mass index; HbA1c, glycosylated hemoglobin; BP, blood pressure.
[a]HbA1c was measured in 78% of the PB/ELBW group and 96% of the control group.
[b]Baseline diameter of the brachial artery.

TABLE 2 | Brachial artery measurements in young adults born very preterm or with extremely low birthweight and term-born controls.

	PB/ELBW *N* = 50	95% CI	Controls *N* = 49	95% CI	*P*-value
Baseline diameter (mm), mean	3.21	(3.07, 3.35)	3.37	(3.19, 3.54)	0.17
Peak diameter at FMD (mm), mean	3.39	(3.24, 3.54)	3.61	(3.44, 3.77)	0.57
Peak diameter at NID (mm), mean	4.02	(3.85, 4.18)	4.15	(4.00, 4.31)	0.23
Absolute FMD (mm), mean[a]	0.17	(0.14, 0.21)	0.24	(0.20, 0.28)	0.01
Absolute NID (mm), mean[a]	0.79	(0.73, 0.86)	0.79	(0.74, 0.83)	1.00
Percentage FMD (%), mean	5.4	(4.2, 6.6)	7.5	(6.2, 8.9)	0.02
Percentage NID (%), mean	25.0	(22.8, 27.2)	24.5	(22.2, 26.8)	0.75
FMD/NID (%), mean	20.3	(16.1, 24.5)	30.8	(25.9, 35.6)	0.001

PB/ELBW, very preterm born (<29 weeks of gestation) or with extremely low birthweight (<1,000 g); FMD, flow-mediated dilatation; NID, nitroglycerine induced dilatation.
[a]Adjusted for baseline diameter.

CI 4.2, 6.6) vs. 7.5% (95% CI 6.2, 8.9), respectively ($p = 0.02$). The absolute brachial artery diameters at baseline, after FMD and after NID, are shown in **Figure 3** and the percentage FMD in **Figure 4**.

No confounding effect was found for sex, age, BMI, HbA1c level, smoking status, or blood pressure on baseline-adjusted difference in absolute FMD between PB/ELBWs and controls ($p = 0.56$, $n = 83$).

Nitroglycerine-Induced Dilatation (NID)

There was no significant difference in absolute and percentage NID between the PB/ELBW group and the control group. Mean absolute NID was 0.79 mm (95% CI 0.73, 0.86) in the PB/ELBW group and 0.79 mm (95% CI 0.74, 0.83) in the control group ($p = 1.0$); mean percentage change was 25.0% (95% CI 22.8, 27.2) and 24.5% (95% CI 22.2, 26.8), respectively ($p = 0.74$) (**Table 2**).

Endothelial-Dependent Dilatation Capacity (FMD Capacity)

FMD relative to maximal dilatation capacity measured by NID was significantly lower in the PB/ELBW group compared with the control group. The mean FMD/NID was 20.1% (95% CI 16.1, 24.5) and 30.8% (95% CI 25.9, 35.6), respectively ($p = 0.001$), shown in **Table 2** and **Figure 5**.

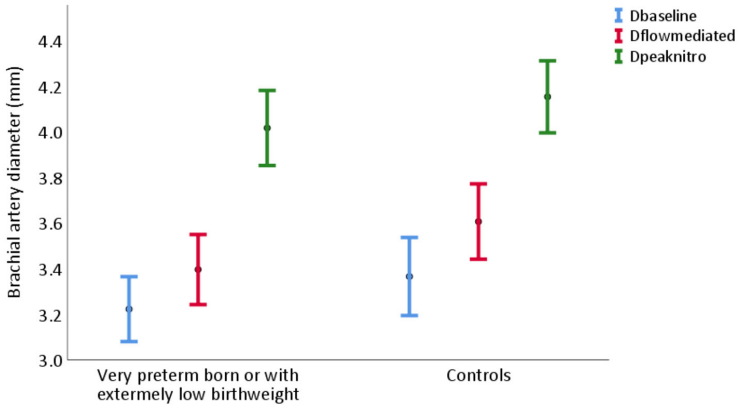

FIGURE 3 | Brachial artery diameter at baseline (blue), after flow-mediated dilatation (red), and after nitroglycerine induced dilatation (green) in young adults born very preterm or with extremely low birthweight (PB/ELBW) and term-born controls, presented as mean with 95% CI. Absolute flow-mediated dilatation was 0.17 mm (95% CI 0.14, 0.21) in the PB/ELBW group and 0.24 mm (95% CI 0.20, 0.28) in the control group. Dbaseline: brachial artery diameter at baseline; Dflowmediated: brachial artery diameter after flow-mediated dilatation; Dpeaknitro: brachial artery diameter after nitroglycerine induced dilatation.

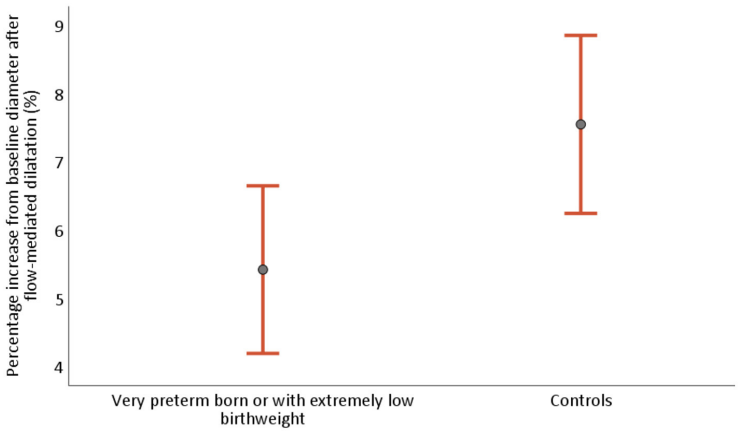

FIGURE 4 | Percentage flow-mediated dilatation (FMD) of the brachial artery from baseline in young adults born very preterm or with extremely low birthweight (PB/ELBW) and term-born controls, presented as mean with 95% CI. Percentage FMD was 5.4% (95% CI 4.2, 6.6) for PB/ELBW and 7.5% (95% CI 6.2, 8.9) for controls.

FMD by Cohort and by Subgroup of Birthweight and GA

For cohorts 1, 2, and 3, the mean age at assessment was 34 (SD 1.5) years, 27 (SD 0.6) years, and 20 (SD 0.9) years, median GA 28 (IQR 2) weeks, 27 (IQR 3) weeks, and 27 (IQR 1) weeks, and mean birthweight 1,022 (SD 218) g, 942 (SD 238) g, and 885 (SD 208) g, respectively. There was a non-significant tendency of lower FMD in the older cohorts among controls, but we found no difference in absolute or percentage FMD, or FMD capacity, between the three cohorts of PB/ELBW (p = 0.6, 0.8, and 0.3, respectively). Characteristics and FMD measures by cohort are shown in **Table 3** and **Figure 6**.

We also compared subgroups of PB/ELBW with the lowest birthweight and GA with the remaining participants of the PB/ELBW group. We found no significant difference in mean absolute or percentage FMD or FMD capacity between the subgroup with birthweight ≤800 g or GA ≤26 (38% of PB/ELBW) and the remaining PB/ELBW group (p = 1.0, 1.0, and 1.0, respectively), or between the subgroup with birthweight ≤700 g or GA ≤25 (22% of PB/ELBW)

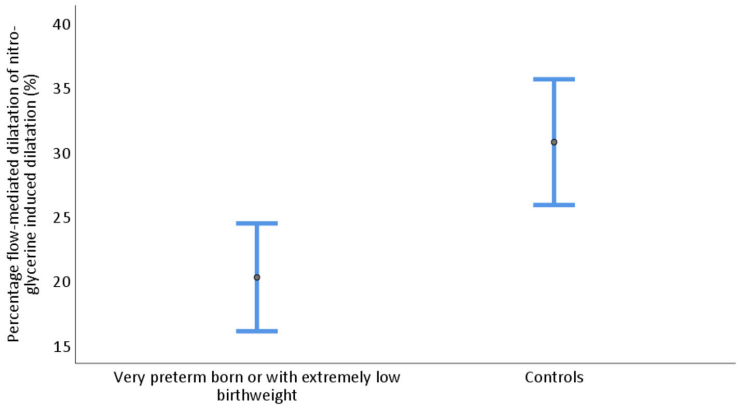

FIGURE 5 | Endothelial-dependent dilatation capacity measured as flow-mediated dilatation in percent of nitroglycerine induced dilatation of the brachial artery in young adults born very preterm or with extremely low birthweight (PB/ELBW) and term-born controls presented as mean with 95% CI. Endothelial-dependent capacity was 20.1% (95% CI 16.1, 24.5) for PB/ELBW and 30.8% (95% CI 25.9, 35.6) for controls.

TABLE 3 | Characteristics of participants born very preterm or with extremely low birthweight, by cohort.

	Cohort born 1982–1985 *n* = 21	Cohort born 1991–1992 *n* = 16	Cohort born 1999–2000 *n* = 13	*P*-value
Age (years), mean (SD)	34 (1.5)	27 (0.6)	20 (0.9)	< 0.01
BW (g), mean (SD)	1,022 (218)	942 (238)	885 (208)	0.2
GA (weeks), median (IQR)	28 (2)	27 (3)	27 (1)	0.3
BMI (kg/m^2), mean (SD)	24.8 (4.1)	24.6 (3.7)	21.8 (4.5)	0.1
Height (cm), mean (SD)	169.0 (7.5)	169.2 (9.0)	166.7 (8.5)	0.7
Weight (kg), mean (SD)	71.1 (13.7)	70.7 (14.1)	61.2 (16.0)	0.1
Smokers, *n*	3	1	0	0.3
HbA1c (mmol/mol), mean (SD)	32.7 (2.8)	32.5 (2.3)	32.9 (2.3)	1.0
Systolic BP (mmHg), mean (SD)	112 (14)	115 (12)	109 (10)	0.4
Diastolic BP (mmHg), mean (SD)	74 (10)	71 (7)	67 (8)	0.1
Baseline diameter (mm), mean (95% CI)	3.33 (3.14, 3.52)	3.09 (2.18, 3.36)	3.18 (2.40, 3.50)	0.3
Absolute FMD (mm), mean (95% CI)	0.20 (0.14, 0.26)	0.16 (0.08, 0.23)	0.16 (0.08, 0.25)	0.6
Percentage FMD (%), mean (95% CI)	5.8 (4.0, 7.7)	5.0 (2.6, 7.4)	5.2 (2.3, 8.2)	0.8
FMD/NID (%), mean (95% CI)	24.1 (17.1, 31.2)	16.4 (9.7, 23.4)	18.5 (9.4, 27.7)	0.3

BW, birthweight; GA, gestational age; IQR, interquartile range; BMI, body mass index; HbA1c, glycosylated hemoglobin; BP, blood pressure; FMD, flow-mediated dilatation; NID, nitroglycerine-induced dilatation.

and the remaining PB/ELBW group (p = 0.6, 0.7, and 0.3, respectively).

FMD by Sex

In analyses of absolute (baseline-adjusted) and percentage FMD separated by sex, we found significantly lower FMD in the female PB/ELBW group compared with the control group, but no significant difference among men. However, the interaction analysis did not show a significant sex effect on the total group difference in absolute FMD ($p = 0.18$). In women, mean absolute FMD was 0.15 mm (95% CI 0.09, 0.20) in the PB/ELBW group vs. 0.25 mm (95% CI 0.21, 0.30) in the control group ($p = 0.004$), with mean percentage change 5.0% (95% CI 3.1, 7.0) vs. 8.6% (95% CI 7.0, 10.3), respectively ($p = 0.01$). In men, mean absolute FMD was 0.20 mm (95% CI 0.15, 0.26) in the PB/ELBW group vs. 0.23 mm (95% CI 0.15, 0.30) in the control group ($p = 0.3$), with mean percentage change 5.8% (95% CI 4.2, 7.4) vs. 6.0% (95% CI 3.9, 8.1), respectively ($p = 0.9$) (shown in **Figure 7**). The difference in absolute FMD diameter change between PB/ELBW and controls was 0.07 mm (95% CI −0.03, 0.18) greater in the female participants vs. the male participants (p = 0.18).

DISCUSSION

Among 50 young adults with mean age 28 (SD 6) years born preterm at median GA 27 (IQR 2) weeks, and with mean

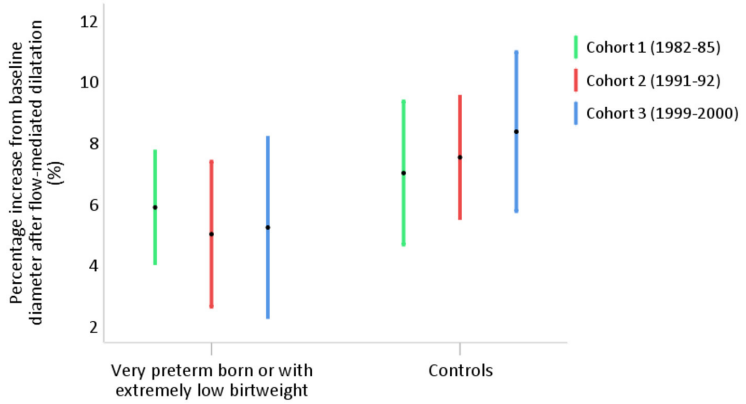

FIGURE 6 | Percentage flow-mediated dilatation of the brachial artery in young adults born very preterm or with extremely low birthweight (PB/ELBW) born in year 1982–1985 (cohort 1, $n = 21$), 1991–1992 (cohort 2, $n = 16$), and 1999–2000 (cohort 3, $n = 13$), presented as mean with 95% CI. Percentage FMD was 5.8% (95% CI 4.0, 7.7) in cohort 1, 5.2% (95% CI 2.6, 7.4) in cohort 2, and 5.2% (95% CI 2.3, 8.2) in cohort 3.

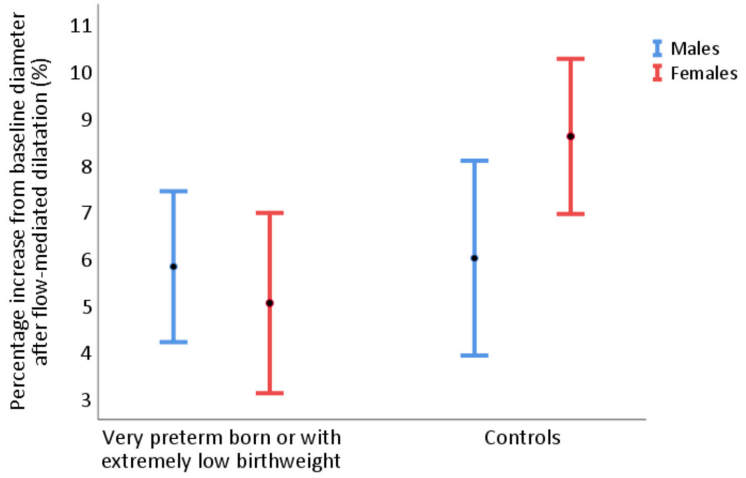

FIGURE 7 | Percentage flow-mediated dilatation (FMD) of the brachial artery in 24 men and 26 women born very preterm or with extremely low birthweight, and term-born controls (20 men and 29 women), presented as mean with 95% CI. In women, percentage FMD was 5.0% (95% CI 3.1, 7.0) for PB/ELBW vs. 8.6% (95% CI 7.0, 10.3) for controls. In men, percentage FMD was 5.8% (95% CI 4.2, 7.4) for PB/ELBW vs. 6.0% (95% CI 3.9, 8.1) for controls.

birthweight 961 (SD 225) g, we found reduced flow-mediated dilatation and endothelial-dependent dilatation capacity of the brachial artery, compared with term-born controls.

Similar to our study, Bassareo et al. (29) found reduced endothelial function measured by finger plethysmography in young adults with mean GA 27.8 (SD 2.2) weeks and mean birthweight 838 (SD 116) g. In addition, Martin et al. (24) found reduced microvascular endothelial function in a small group of school children with mean age 9 (SD 1.4) years and with mean birthweight 2,188 (SD 224) g. Contrary to our findings, Flahault et al. (28), Singhal et al. (26), and Hovi et al. (27) reported normal flow-mediated dilatation of the brachial artery in young adults born preterm or with LBW. Flahault's study included 85 individuals born preterm with mean GA 27.2 (SD 1.4) weeks and birthweight 963 (SD 225) g, similar to our population, whereas the populations in the studies by Singhal and Hovi had higher GA and birthweight [mean GA 31 (SD 2.7) and 29.5 (SD 2.4) weeks, respectively, and mean birthweight 1,400 g (SD 300 g

and 112 g, respectively)]. In addition, Shingal's study population was younger with mean age 15 (SD 0.9) years at the time of assessment. Higher GA and birthweight, and younger age at time of assessment, could explain the differences in results in these two studies, compared with the present study.

Previous studies have showed an inverse association between GA and birthweight and the risk of cardiovascular disease (2, 5). We were not able to show a significant difference in FMD or endothelial-dependent dilatation capacity between the subgroups of PB/ELBW with the lowest birthweight and GA, compared with the remaining PB/ELBW group. Similarly, we found no difference in FMD or endothelial-dependent dilatation capacity between the three PB/ELBW cohorts. While the lower mean birthweight in the youngest cohort compared with the oldest cohort (885 g vs. 1,022 g) could lead to a lower FMD, the younger age at the time of assessment and possible improvement in neonatal intensive care treatment the last decades could have the opposite influence on FMD in the younger cohorts. However, the study samples within the subgroups and cohorts of the PB/ELBWs were too small to show minor group differences.

A complex interplay between circulating sex steroid hormones and steroid-independent mechanisms are assumed to contribute to a stated overall difference in endothelial function between healthy young men and women (19). Both sexes show an age-dependent decline in endothelial function (19), and a steeper decline is seen in postmenopausal women (38). This claimed sex difference was, however, doubted by Atkinson et al., who argued that the reported sex differences in fact may be a result of calculation bias because of baseline diameter variations (39, 40). We found a small non-significant difference in mean baseline diameter between the PB/ELBW and control group, and chose to compare the absolute brachial artery diameter changes adjusted for baseline diameter. Fewer men (44%) than women participated in the study, and the sex distribution within each group was slightly unequal. The PB/ELBW group consisted of 48% men and the control group of 41% men. We did, however, not find any sex differences for the main result (absolute FMD in PB/ELBW and controls), although the small sample size provides little statistical power for the interaction analysis.

Most studies assessing FMD of the brachial artery present their results as percentage diameter change from baseline. A cut-off value for FMD indicating normal endothelial function in young adults is not established. The FMD results differ widely between studies with seemingly similar populations, and mean FMD values across populations range from −1.9 to 19.2% (41). Divergent FMD results are partly attributable to methodology and technical aspects of the measurements of the brachial artery. The FMD response may differ with duration, degree, and location of occlusion of the brachial artery (41), with choice of artery, or with method to induce stress to the artery. Possible diurnal variations of FMD may also exist (42, 43). A large Japanese multicenter study proposed a cut-off value for FMD indicating normal vascular function of 8.9% in subjects younger than 40 years and 7.1% independent of age (44). In the present study, the mean FMD in the PB/ELBW group was 5.4%; in the control group, 7.5%. Among the 50 participants in the PB/ELBW group and 49 participants in the control group, 76 and 65% had FMD lower than 8.9% in the respective groups, whereas 66 and 47% had FMD lower than 7.1%.

The interrelationship between endothelial-dependent vasodilatation and endothelial-independent NID and the relation to cardiovascular risk factors and disease are debated (45–48). Impaired NID might reflect vascular smooth muscle cell dysfunction and vascular structure alternations due to atherosclerosis (49, 50). The presence of multiple cardiovascular risk factors or established cardiovascular disease has previously been reported to impair NID of the brachial artery (46).

While a low FMD/NID ratio could indicate cardiovascular risk factors impairing the endothelial function, but no established vascular disease, individuals with established vascular disease and vascular smooth cell dysfunction might have normal or even high FMD/NID ratio despite impaired endothelial function. Assessed together, however, FMD and FMD/NID ratio might give valuable information.

The term of describing endothelial function as endothelial-dependent FMD capacity compared with maximal dilatation capacity, in terms of endothelial-independent NID, is probably most valuable when NID is normal and can be estimated as maximal dilatation. Maruhashi et al. suggested an age-independent cut-off value of 15.6% for NID of the brachial artery in healthy Japanese people (44). We found mean NID of 25% in both the PB/ELBW and control group. Only 8% in the PB/ELBW group and 14% in the control group had NID <15.6%. None of these were smokers, and all had normal values for HbA1c and normal resting blood pressure. Four (one in the PB/ELBW group) were overweight with BMI >25 kg/m^2. Normal NID response in most of our study participants corresponds well to the population characteristics with a mean age of 28 years, and no established atherosclerotic disease or multiple cardiovascular risk factors. We found decreased endothelial-dependent dilatation capacity in the PB/ELBW compared with controls, 20% vs. 31%, respectively, indicating an impairment of the endothelial function and normal vascular smooth cell function.

The increasing group of adults born very preterm carry a risk of a range of long-term complications and early adult death (2). Although large efforts are invested to facilitate the survival of these infants, the investments in long-term follow-up have been comparatively marginal, and the knowledge on life-long health prospects is sparse. It is especially important to detect modifiable risk factors or early signs of disease in this group, to reduce the life-long disease burden to both individuals and society.

Both indices of microvascular and macrovascular endothelial dysfunction are associated with risk of cardiovascular disease; however, the different methods used to evaluate endothelial function are not interchangeable, and the results may represent different aspects of cardiovascular risk (51). Only a few previous studies have investigated endothelial function in young adults born very preterm and with extremely low birthweight, and to our knowledge, only the study by Flahault et al. (28) investigated macrovascular endothelial function by FMD in this patient group. Our study, therefore, adds important information to

the knowledge of the health and future risk of young adults born preterm.

Although dependent on examinator skills, FMD is a preferable method to assess macrovascular endothelial function because it is non-invasive, painless, requires equipment available at any cardiology department, and has a short examination time of about 30 min. It is a well-known method with widespread use in research.

Previous studies have reported an association of traditional cardiovascular risk factors with preterm birth and LBW (6–10), corresponding to an increased risk of cardiovascular disease in the group (2, 18). Our findings of reduced endothelial function in the PB/ELBW group compared with the control group, with no difference in other known risk factors (smoking, BMI, diabetes, hypertension), suggest that extremely low birthweight and very preterm birth might represent individual risk factors for endothelial dysfunction, and thus cardiovascular disease. This association may be due to complex mechanisms triggered already in the intrauterine and neonatal period. Infections and inflammatory processes during pregnancy are major risk factors for premature birth, and might itself give an increased cardiovascular risk (52, 53). Most very preterm born neonates have been exposed to extensive intensive care treatment and long periods of oxygen supplementation that can induce oxidative stress reactions, and might be harmful in a long-term perspective (54). In addition, behavioral issues associated with increased cardiovascular risk, like physical inactivity, may affect cardiovascular health, and previous studies have reported reduced level of activity and physical fitness in preterm born children and adults (55, 56). Maternal smoking or exposure to second-hand smoking during pregnancy (57), and maternal diabetes (58) are associated with preterm delivery and also with risk factors of cardiovascular disease in offspring. There is, however, little evidence that these exposures represent individual risk factors for offspring cardiovascular disease (59, 60). Further studies are needed to investigate the causal pathways of the associations between preterm birth or low birthweight and cardiovascular risk.

The study was part of a regional population-based cohort study of individuals born PB/ELBW and individually age- and sex-matched term-born controls recruited to long-term follow-up since year 2000. The study design, and similarity of the PB/ELBW and control group regarding characteristics and cardiovascular risk factors at the time of the assessment reduce the risk of confounding. The method of measuring endothelial function is well-established (20, 61). To diminish procedure-related variance, the measurements were performed by a highly trained single sonographer with strict adherence to the International Brachial Artery Reactivity Task Force, and the calculated interrater variability was excellent (62).

There were limitations to this study. Only 34% of the eligible PB/ELBW subjects were examined. The PB/ELBWs who did not participate had similar median GA and mean days of ventilatory support and oxygen demand, compared with those who participated (**Table 4**). The subjects who did not participate had a slight and non-significant lower mean birthweight (896 g vs. 961 g, $p = 0.08$), and a higher occurrence of moderate or severe bronchopulmonary dysplasia (63) compared with the PB/ELBWs who participated. Flahault et al. described an association between bronchopulmonary dysplasia and altered elastic properties of the brachial artery, but found no similar association with impaired FMD (28). The possible selection bias could, however, most likely lead to an underestimation of the difference in FMD between the PB/ELBW and the control group. Although the PB/ELBW and control group were well-matched for several known cardiovascular risk factors, we had no information regarding cholesterol levels or family history of cardiovascular disease. Previous studies have found no association between maternal familial hypercholesterolemia and preterm birth or LBW (64), but a possible association of preterm birth with dyslipidemia (65). A small risk of unknown confounding cannot be ruled out. However, as all study participants were normotensive and all measurements of HbA1c were well beneath the cut-off for prediabetes, and the BMI was similar in the two groups, there is in our opinion not a high risk of lipid discrepancy between the groups. The measurements

TABLE 4 | Characteristics of participants and non-participants born very preterm or with extremely low birthweight.

	Participants ($n = 50$)	**Non-participants ($n = 98$)**	***P*-value**
Male/female, n	24/26	50/48	0.08
Cohort 1/2/3, n	21/16/18	28/19/51	
BW, g, mean (SD)	961 (225)	896 (178)	
GA, weeks, median (IQR)	27 (2)	27 (2)	0.3
Days on ventilator, mean (SD)	8 (10)	8 (9)	0.9
Days on CPAP, mean (SD)	28 (14)	31 (19)	0.6
Days on O_2 supplement, mean (SD)	51 (37)	57 (42)	0.4
Moderate/severe BPD, *n*	28%	45%	0.04
Prenatal steroid treatment, *n*	48%	60%	0.3
Postnatal steroid treatment, *n*	18%	25%	0.3
Surfactant, *n*	40%	52%	0.2

Cohort 1, born 1982–1985; cohort 2, born 1991–1992; cohort 3, born 1999–2020; BW, birthweight; GA, gestational age; IQR, interquartile range; CPAP, continuous positive airway pressure; O_2, oxygen; BPD, bronchopulmonary dysplasia (classified as moderate/severe BPD if need for supplemental O_2 or CPAP at 36 weeks of gestation).

of HbA1c were incomplete. However, the BMI was normal in the 13 study participants with missing HbA1c (mean BMI was 21.8 kg/m^2), indicating a low risk of metabolic syndrome in these subjects.

In conclusion, the young adults born very preterm (<29 weeks of gestation) or with extremely low birthweight (<1,000 g) had lower flow-mediated vasodilatation and reduced endothelial-dependent dilatation capacity of the brachial artery compared with term-born controls. Reduced FMD is suggestive of vascular endothelial dysfunction, and well-established as an early marker for development of atherosclerotic cardiovascular disease. Our findings suggest that very preterm birth and extremely low birthweight may be independent risk factors for cardiovascular disease. We therefore recommend follow-up programs for these children to extend into adulthood, with special attention to reduce modifiable risk factors for atherosclerotic cardiovascular disease, and increase awareness of early symptoms of cardiovascular disease in adults born very preterm.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by the Regional Committee for Medical and Health Research Ethics of the Western Norway Health Authority (REC 2017/0068). The patients/participants provided their written informed consent to participate in this study.

AUTHOR CONTRIBUTIONS

BE conceived and designed the analysis, collected and organized data, carried out the analyses, drafted the initial article, and revised the article. ME coordinated data collection, organized data, and critically reviewed the article for important intellectual content. GG conceptualized and designed the study and critically reviewed the article for important intellectual content. MV provided funding, designed the data collection instruments, coordinated and supervised data collection, and critically reviewed the article for important intellectual content. KH gave advice on the analysis of data, has participated in the interpretation of data, and has critically reviewed the article for important intellectual content. EL conceptualized and designed the study and critically reviewed the article for important intellectual content. All authors approved the final article and take responsibility for all aspects of reliability of the data presented and their discussed interpretation.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fped.2021.734082/full#supplementary-material

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Myocardial function including estimates of myocardial work in young adults born very preterm or with extremely low birthweight - a cohort study

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Abstract

Background Preterm birth and low birthweight have been associated with increased risk of heart failure and cardiovascular disease in young adulthood. However, results from clinical studies of myocardial function are not consistent. Echocardiographic strain analyses allow detection of early stages of cardiac dysfunction, and non-invasive estimates of myocardial work can provide additional information on cardiac function. We aimed to evaluate left ventricular (LV) myocardial function including measures of myocardial work in young adults born very preterm (gestational age < 29 weeks) or with extremely low birthweight (< 1000 g) (PB/ELBW), compared with term-born age- and sex matched controls.

Methods 63 PB/ELBW and 64 controls born in Norway in the periods 1982–1985, 1991–1992, and 1999–2000 were examined with echocardiography. LV ejection fraction (EF) and LV global longitudinal strain (GLS) were measured. Myocardial work was estimated from LV pressure-strain loops after determination of GLS and construction of a LV pressure curve. Diastolic function was evaluated by determination of the presence or absence of elevated LV filling pressure, including measures of left atrial longitudinal strain.

Results The PB/ELBW with mean birthweight 945 (standard deviation (SD) 217) grams, mean gestational age 27 (SD 2) weeks, and mean age 27 (SD 6) years had LV systolic function mainly within normal range. Only 6% had EF < 50% or impaired GLS >-16%, but 22% had borderline impaired GLS between − 16% and − 18%. Mean GLS in PB/ELBW (-19.4% (95% confidence interval (CI) -20.0, -18.9)) was impaired compared to controls (-20.6% (95% CI -21.1, -20.1)), p = 0.003. Lower birthweight was associated to more impaired GLS (Pearson correlation coefficient − 0.2). Means of EF, measures of diastolic function including left atrial reservoir strain, global constructive and wasted work, global work index and global work efficiency was similar in PB/ELBW and controls.

Conclusion The young adults born very preterm or with extremely low birthweight had impaired LV-GLS compared to controls, although systolic function mainly within normal range. Lower birthweight was associated with more

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impaired LV-GLS. These findings could indicate an elevated lifetime risk of developing heart failure in preterm born individuals. Measures of diastolic function and myocardial work were similar compared to controls.

Keywords Speckle-tracking echocardiography, Myocardial work, Very preterm, Extremely low birthweight

Background

Due to improvement in neonatal intensive care over the last decades neonates born extremely preterm (<28 weeks of gestation) now have survival rates>80% [1–3], thus representing approximately 0.5% of all children growing up in Norway [4]. Preterm birth and low birthweight are associated with increased prevalence of cardiovascular risk factors [5] and a higher risk of cardiovascular disease, all-cause mortality, and young adult death [2, 6–11]. Gestational age (GA) and birthweight (BW) are found to be inversely associated with increased risk of cardiovascular disease and all-cause mortality [2, 11], but an increased risk has been described even in individuals born only moderate to late preterm (32–37 weeks of gestation) [2, 11].

Large register-based studies have found increased risk of heart failure in adolescents and young adults born preterm [8, 12], but clinical studies of myocardial function measured by echocardiography or cardiac magnetic resonance imaging have reported diverging results. Several studies have reported mainly preserved left ventricle (LV) ejection fraction (EF), but impaired LV myocardial strain, diastolic function and myocardial response to physical stress [13–17]. However, LV myocardial strain patterns have also been described as normal or even hypercontractile [18].

Traditionally, definitions of, and guidelines regarding management of heart failure have been based on measures of EF. In later years, echocardiographic speckle-tracking derived strain analysis has allowed detection of subclinical early stages of LV dysfunction, and its role in risk stratification is increasing [19]. Additionally, although not yet much used in clinical practice, analyses of non-invasively measured LV myocardial work (MW) to describe myocardial function have been introduced as an advancement of LV strain analyses, due to reduction of the strain analysis` load dependent limitations [20, 21]. These measures strongly correlate to invasively measured MW [22, 23]. New parameters describing diastolic myocardial function have also been introduced. Analysis of left atrial (LA) reservoir function measured by LA peak longitudinal strain has been reported to detect increased LV filling pressure and LV diastolic dysfunction at an earlier point than traditional echocardiographic diastolic parameters [24, 25] like mitral inflow and annular tissue velocities and LA volumes. Measures of LA reservoir function is reported to be of clinical and prognostic relevance especially concerning heart failure with preserved EF [26]. To our knowledge MW and LA strain have not yet been investigated in preterm born children or young adults.

We had the opportunity to investigate myocardial function in a cohort of young adults born very preterm or with extremely low birthweight, who have been followed since birth by the Project Extreme Prematurity in Bergen, Norway. The aim of this study was to investigate the impact of preterm birth and low birthweight on left ventricular myocardial function at young adult age, by adding new echocardiographic parameters such as MW and LA longitudinal strain to measures of LV EF, LV longitudinal strain and traditional echocardiographic measured markers of diastolic function.

Materials and methods

Study design and participants

The Project Extreme Prematurity, organized by the WestPaed Research group in Bergen, Norway, has included three regional cohorts of in total 153 young adults born very preterm or with extremely low BW (PB/ELBW), and in total 139 individually age and sex matched term-born controls. Inclusion criteria were birth at GA<29 weeks or extremely low BW<1000 g. Subjects born in 1982–1985 (cohort 1) and in 1991–1992 (cohort 2) were retrospectively recruited, while those born in 1999–2000 (cohort 3) were prospectively recruited. The inclusion and longitudinal follow-up of these cohorts have been described in detail previously [27, 28]. In total 6 individuals were lost to follow-up; 1 control and 2 PB/ELBW because of death and 3 PB/ELBW due to severe disability.

During the period 2017–2020 the participants were invited to a third follow-up, including a first-time echocardiographic examination. A total of 127 participants were examined, including 63 PB/ELBW subjects (43% men) and 64 controls (44% men) (See flow chart of study participation in Additional file 1). Of the participants, 49 belonged to cohort 1, 37 to cohort 2, and 41 to cohort 3.

The Regional Committee for Medical and Health Research Ethics of the Western Norway Health Authority approved the study (REC 2017/0068). Written informed consent was obtained from all participants.

Echocardiographic assessment

All echocardiographic examinations were performed on a Vivid E9 ultrasound system (GE Healthcare, Horten, Norway) using a 5Sc (1.5–4.6 MHz) or equivalent 4Vc-D (1.4–5.2 MHz) transducer for all imaging. All images were saved as three consecutive cardiac cycles during sinus rhythm, on external hard drives allowing offline

analyses using the echocardiographic software, EchoPAC version 204 (GE Healthcare, Horten, Norway). All participants underwent a comprehensive functional echocardiographic examination, which included grayscale images optimized for 2-dimensional (2D) speckle analysis. The images were analysed according to recommendations from the European and American Society of Echocardiography [29–31] and as proposed by Russel et al. [23] regarding analyses of MW.

LV EF was calculated using the modified Simpson biplane technique and from long-axis 2-dimentional (2D) measurements using the Teicholz formula. LV wall thickness, LV internal diameter and myocardial mass index (calculated using the Devereux formula and indexed to body surface area (BSA)) were calculated using long-axis end-diastolic 2D measurements. Diastolic function was evaluated as recommended by determination of the presence or absence of elevated LV filling pressure [31], and was measured by pulse waved Doppler (early/late mitral inflow velocity (E/A)), continuous wave Doppler (tricuspid valve regurgitation jet peak gradient), tissue Doppler (mitral septal and lateral annular early diastolic velocity (e´) and E/e´), and measures of LA end-systolic volume in four chamber view indexed by BSA.

Gray scale images for 2D speckle-tracking analysis were acquired at frame rate (frames/second) to heart rate (beats/minute) ratio 0.7–0.9. Longitudinal strain curves from one ventricular cycle from standard apical four, three and two chamber view images, analysed for 18 subsegments, were used for LV longitudinal strain assessment. For LA longitudinal strain assessment, we used longitudinal strain curves from one atrial cycle from apical four chamber view images, with zero reference point set at the start of the ECG R-wave.

Speckle-tracking of the LV and the LA were performed using automated function imaging (AFI). Visual inspection ensured that the region of interest (ROI) included the whole of the myocardium from the atrioventricular valve annulus to the LV apex or LA roof and from the endocardial border to the epicardial border. If the automatic tracking was not satisfactory, the ROI was adjusted manually until the best possible tracking was obtained. LV tracking compromised by artefacts/shadows or image acquisitions with less than five of six appropriately tracked segments per apical view were excluded. Peak systolic longitudinal strain was measured at aortic valve closure for LV measures and at end-systole for LA measures. MW was calculated by the EchoPAC software after determination of LV peak systolic global longitudinal strain (LV-GLS) and construction of a LV pressure curve. The LV pressure curve was derived from non-invasive brachial blood pressure (BP) measures and the timing of the cardiac cycles` isovolumetric and ejection phases, which were defined by opening and closure of the mitral valve. The area of the LV pressure–strain loop represents MW. Since work by definition equals force times length, the use of pressure and strain does not provide a direct measure of work, but can be used as a valid approximation of it as the method is found to strongly correlate to invasively measured MW [22, 23]. Global MW obtained from LV-GLS with less than 17 LV segments, due to suboptimal tracking or image quality, were excluded. The timing of the aortic and mitral valve opening and closure was decided using continuous-waved and pulse-waved Doppler, respectively, and confirmed by visualizing the opening and closure of the valves from the LV three chamber apical view.

In this paper, LV-GLS describes the average peak systolic longitudinal strain from all three left ventricular apical views, and LV-4 C-LS is used to describe the peak systolic LV longitudinal strain from the apical four chamber view (Fig. 1).

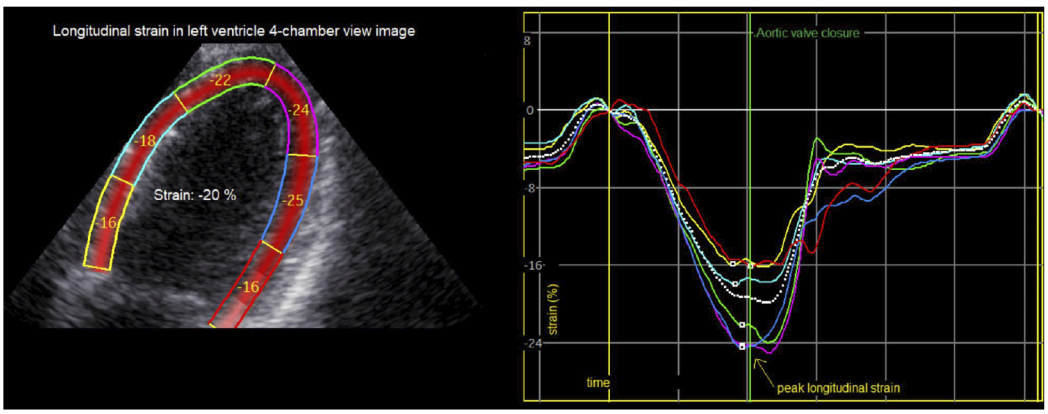

Fig. 1 Left ventricle peak longitudinal strain measures generated from apical 4-chamber view image

LA reservoir, conduit, and contractile strain generated from LA longitudinal strain analyses from the apical four chamber view images are used to describe LA reservoir function during LV systole, conduit function during early diastole, and booster pump function during late diastole (Fig. 2). All longitudinal strain values are described as negative percentages, as they describe myocardial shortening of segments relative to original length, except LA-reservoir and conduit strain which represent myocardial lengthening relative to original length and therefore are described as positive percentages.

Myocardial global constructive work (GCW), which contributes to ejection, is used to describe the mean net effect resulting from positive LV work (shortening) during systole plus negative LV work (lengthening) during isovolumetric relaxation in all 18 LV segments. Myocardial global wasted work (GWW), which does not contribute to ejection, is used to describe the mean net effect resulting from negative LV work during systole plus positive LV work during isovolumetric relaxation in all 18 LV segments. Global work index (GWI) is used to describe total performed LV work from the opening to the closure of the mitral valve, and global work efficiency (GWE) is used to describe global constructive work relative to the total of global constructive and waste work (GWE=GCW/(GCW+GWW)). MW values are described as pressure percentages (mmHg%) as they are derived from LV pressure-strain loop analysis (Fig. 3).

EF Simpson and EF Teicholz ≥50% were considered normal and EF<50% considered reduced. LV-GLS >-16% was considered abnormal, -16% to -18% borderline normal, and <-18% normal [32, 33]. Abnormal values of conventional LV diastolic parameters were defined as septal or lateral e´ <0.07 m/s or <0.10 m/s, respectively, average E/e´ ratio>14 (using the average of septal and lateral e´), peak tricuspid valve regurgitation jet velocity peak gradient>30 mmHg, and LA volume index>34 ml/m^2 /s [34].

Mean reference values of measures of MW are former described in the NORRE study as GWI 1896 mmHg%,

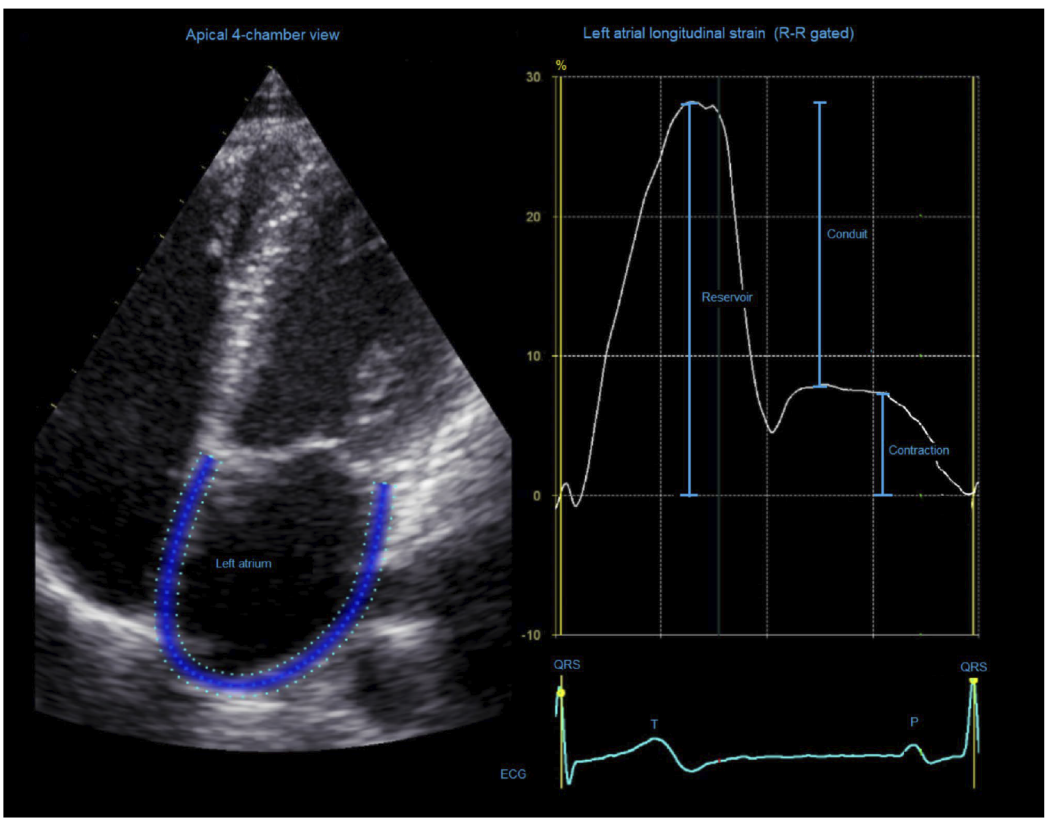

Fig. 2 Left atrial reservoir, conduit, and contractile function measured by left atrial longitudinal strain in an apical 4-chamber view image

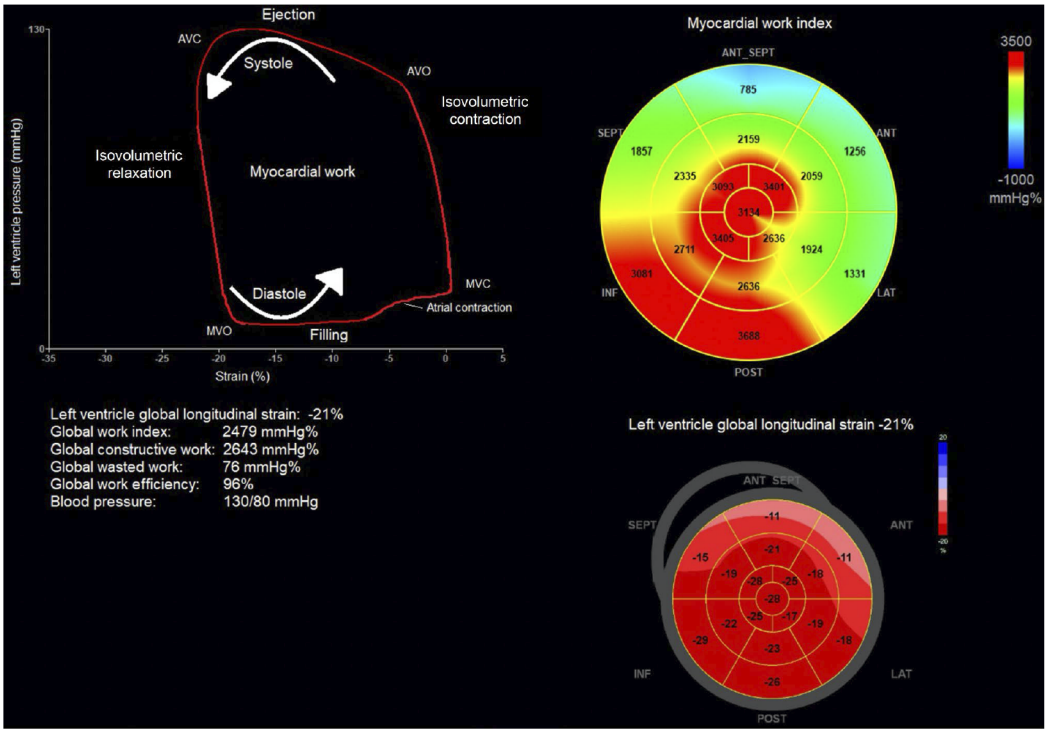

Fig. 3 Myocardial work (MW) was calculated using EchoPAC version 204 (GE Healthcare) after determination of left ventricle (LV) peak global longitudinal strain and construction of a LV pressure curve. The LV pressure curve was derived from non-invasively measured brachial pressure and timing of the cardiac cycles` isovolumetric and ejection phase defined by mitral valve opening and closure. The area of the LV pressure–strain loop was defined as MW AVC: aortic valve closure, AVO aortic valve opening, MVO: mitral valve opening, MVC: mitral valve closure

GCW 2232 mmHg%, GWW 78.5 mmHg% and GWE 96% [21, 35].

Mean reference values of LA strain are previously described as 39% for reservoir strain, 23% for conduit strain, and −17% for contractile stain based on findings in a meta- analysis which included both ECG p-wave (P-P gated) or QRS complex (R-R gating) as initiation of LA stain calculations [36].

All imaging and analyses were performed by a single experienced cardiologist (B.E). A random sample of 10 study participants were obtained for inter- and intra-rater analyses. Another experienced cardiologist (T.O) performed the re-analysis for inter-rater reliability. Both were blinded to group affiliation (PB/ELBW or control) and to the original results during re-analyses.

Inter-rater reliability measured by a 2-way mixed-effect model for absolute agreement was 0.8 (95% confidence interval (CI) 0.04, 0.9) for EF Teich, 0.9 (95% CI 0.5, 1.0) for LV-GLS, 0.8 (95% CI 0.0, 1.0) for GWI, and 0.8 (95% CI 0.1, 0.9) for LA reservoir strain. Intra-rater reliability measured by a 2-way mixed-effect model for absolute agreement was 0.8 (95% CI 0.2, 0.9) for EF Teicholz, 0.8 (95% CI 0.3, 1.0) for LV-GLS, 1.0 (95% CI 0.8, 1.0) for GWI, and 0.9 (95% CI 0.6, 1.0) for LA reservoir strain.

Blood pressure

BP was calculated as the average of three measurements with an automated oscillometric device (Biolight BLT V6, Biolight Meditech Company, Guangdong, China) at rest in supine position.

Statistical analysis

Descriptive variables are presented as numbers (%) and outcome data as means with 95% CI or standard deviation (SD). The Kolmogorov- Smirnov test was used to test the normality of the outcome variables, and independent samples t-test (with equal variance not assumed) or two samples Kolmogorov-Smirnov test was applied for comparison between groups, as appropriate. For comparison of descriptive data Chi-square test or Fisher`s exact test was applied, as appropriate.

Linear regression analyses were used to identify confounders. Analyses of covariance (ANCOVA) was used for comparison between PB/ELBW and controls for height, weight, and BSA (according to Du Bois formula) adjusted for sex, for LV longitudinal strain adjusted for BSA and echocardiography transducer difference, and for EF, LA volume index, LA strain, and MW parameters adjusted for transducer difference.

Re-analyses were done excluding outliers, but this did not significantly affect the results.

To investigate whether the difference in echocardiographic measures between PB/ELBW and controls differed by sex, an interaction term for sex and group affiliation was added.

Table 1 Characteristics of study participants

	PB/ELBW	Controls	p
Total, n (% males)	63 (43%)	64 (44%)	
Cohort 1 (born 1982–1985), n (%)	23 (37%)	26 (41%)	
Cohort 2 (born 1991–1992), n (%)	19 (30%)	18 (28%)	
Cohort 3 (born 1999–2000), n (%)	21 (33%)	20 (31%)	
GA (weeks), mean (SD)	27 (2)	Term-born	
Birthweight (grams), mean (SD)	945 (217)	3527 (324)	<0.001[a]
Small for gestational age, n (%)	16 (25%)		
Extremely preterm GA < 28 weeks, n (%)	39 (62%)		
Extremely low birthweight < 1000 g, n (%)	37 (59%)		
Moderate/severe BPD, n (%)	21 (33%)		
Days on ventilator, mean (SD)	8 (9)		
Days on CPAP, mean (SD)	33 (14)		
Days on O_2-supplement, mean (SD)	52 (35)		
Pre-natal steroid treatment, n (%)	35 (55%)		
Post-natal steroid treatment, n (%)	14 (22%)		
Surfactant, n (%)	28 (44%)		
Age (years), mean (SD)	27 (6)	28 (7)	0.95[b]
Height (cm), mean (95% CI)	168 (167, 170)	174 (172, 175)	<0.001[c]
Weight (kg), mean (95% CI)	68 (64, 72)	75 (71, 79)	0.01[c]
Smokers, n (%)	4 (6%)	4 (6%)	1.00[b]
BMI (kg/m^2), mean (SD)	24.0 (4.1)	24.9 (6.0)	0.75[b]
BSA (m^2), mean (95% CI)	1.8 (1.7, 1.8)	1.9 (1.8, 1.9)	<0.001[c]
Systolic BP (mmHg), mean (SD)	113 (12)	112 (10)	0.87[b]
Diastolic BP (mmHg), mean (SD)	71 (8)	70 (8)	0.98[b]
Heartrate (beats /minute), mean (SD)	69 (14)	65 (11)	0.13[a]

a = independent samples t test (with equal variance not assumed)

b= non–parametric two sample Kolmogorov– Smirnov test

c= ANCOVA adjusted for sex

PB/ELBW: very preterm born/ with extremely low birthweight, SD: standard deviation, GA: gestational age, BPD: bronchopulmonary dysplasia (classified as moderate/severe BPD if need of supplementary oxygen or CPAP at 36 weeks of gestation), CPAP: continuous positive airway pressure; O_2: oxygen, CI: confidence interval, BMI: body mass index, BSA: body surface area (Du Bois formula), BP: blood pressure, ANCOVA: analysis of covariance

Comparisons of descriptive variables and outcome data between subgroups of study participants were done by Chi-square test or Fisher`s exact test, Welch´s ANOVA or classic one-way ANOVA (with Games-Howell and Tukey`s test used for post hoc analyses, respectively), or by multiple comparison analyses (with Sidak correction) with the same adjustments as described above, as appropriate.

Pearson correlation coefficient (PCC) was used to explore associations between echocardiographic measured myocardial function and descriptive variables. GA was set to 40 weeks in controls when correlations regarding GA was made.

Inter- and intra-rater reliability were measured by a 2-way mixed-effect model for absolute agreement.

All tests were two-sided, and $p<0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

Results

The participants consisted of 63 PB/ELBW subjects and 64 age- and sex matches controls. The PB/ELBW group had significantly lower height and weight, with consequently lower BSA, compared to the control group. There was no significant difference for mean age, body mass index, BP, or smoking status between the groups. The age range was 18–37 years in both the PB/ELBW and the control group. BW in the PB/ELBW group ranged from 450 to 1480 g with mean BW 945 (SD 217) grams. GA ranged from 23 to 34 weeks with mean GA 27 (SD 2) weeks (Table 1). None of the study participants had history of cardiovascular disease.

EF, mitral inflow velocities, and annular tissue velocities were measured in most of the participants (Table 2). Estimation of tricuspid valve regurgitation peak gradient was dependent on the presence of a tricuspid valve regurgitation jet and measurable only in 37 PB/ELBW and 41 controls. Measures of LA strain, MW, and LV strain were limited by image quality and compromised speckle-tracking, and lack of adequately measured BP. LV-4 C-LS was assessed in 61 PB/ELBW and 60 controls, LV-GLS in 50 PB/ELBW and 53 controls, MW in 49 PB/ELBW and 51 controls, and LA strain in 55 PB/ELBW and 55 controls.

Systolic LV function

Mean EF Simpson and EF Teicholz were similar in PB/ELBW and controls, but LV-GLS were significantly impaired in PB/ELBW compared to controls −19.4% (95% CI -20.0, -18.9) versus −20.6% (95% CI -21.1, -20.1) ($p=0.003$) (Table 2; Fig. 4). The difference in LV-GLS between PB/ELBW and controls was not significantly

Table 2 Echocardiographic measures of systolic and diastolic myocardial function in PB/ELBW compared to controls

		PB/ELBW		Controls	P
	n		n		
EF Teicholz (%), mean (95% CI)	63	61 (59, 62)	64	62 (61, 64)	0.18[a]
EF Simpson (%), mean (95% CI)	63	59 (58, 61)	64	61 (59, 62)	0.32[a]
LV-4 C-LS (%), mean (95% CI)	61	-18.7 (-19.2, -18.2)	60	-19.9 (-20.5, -19.4)	0.002[a]
LV-GLS (%), mean (95% CI)	50	-19.4 (-20.0, -18.9)	53	-20.6 (-21.1, -20.1)	0.003[a]
GCW (mmHg%), mean (95% CI)	49	1982 (1901, 2063)	51	2074 (1995, 2153)	0.11[a]
GWW (mmHg%), mean (95% CI)	49	100 (78, 121)	51	92 (71, 113)	0.61[a]
GWI (mmHg%), mean (95% CI)	49	1749 (1666, 1832)	51	1853 (1772, 1934)	0.08[a]
GWE (%), mean (95% CI)	49	95 (93, 96)	51	95 (93, 96)	0.85[a]
IVSDd (cm), mean (95% CI)	63	0.9 (0.8, 0.9)	64	0.9 (0.9, 0.9)	0.08[b]
PWDd (cm), mean 95% CI)	63	0.8 (0.8, 0.8)	64	0.8 (0.8, 0.9)	1.00[b]
LVDd (cm), mean (95% CI)	63	4.6 (4.5, 4.7)	64	4.8 (4.7, 4.9)	0.08[b]
Indexed LV mass (g/m^2), mean (95% CI)	63	69 (65, 73)	64	75 (70, 79)	0.08[c]
Mitral septal e´(m/s), mean (95% CI)	61	0.12 (0.11, 0.12)	63	0.12 (0.11, 0.13)	0.99[b]
Mitral lateral e´ (m/s), mean (95% CI)	62	0.15 (0.14, 0.16)	64	0.16 (0.13, 0.20)	0.96[b]
Average mitral E/e´ratio, mean (95% CI)	61	5.5 (5.3, 5.7)	61	5.4 (5.1, 5.8)	0.82[b]
Mitral E/A-ratio, mean (95% CI)	63	1.5 (1.4, 1.6)	61	1.5 (1.4, 1.6)	0.83[b]
TR peak gradient (mmHg), mean (95% CI)	37	18 (16, 20)	41	16 (15,18)	0.96[b]
LA contraction strain (%), mean (95% CI)	55	-10.5 (-11.5, -9.4)	55	-11.5 (-12.5, -10.3)	0.22[a]
LA conduit strain (%), mean (95% CI)	55	27.5 (25.5, 29.5)	55	28.6 (26.6, 30.6)	0.43[a]
LA reservoir strain (%), mean (95% CI)	55	38.1 (35.7, 40.5)	55	39.7 (37.4, 42.1)	0.34[a]
LA volume (ml/m^2), mean (95% CI)	55	21 (19, 22)	55	21 (19, 22)	0.93[a]

a = ANCOVA adjusted for BSA and transducer difference as appropriate

b= non–parametric two sample Kolmogorov– Smirnov test

c = independent samples t test (with equal variance not assumed)

PB/ELBW: very premature born (<29 weeks of gestation) or with extremely low birthweight (<1000 g), EF: ejection fraction, CI: confidence interval, LV: left ventricle, 4 C-LS: 4-chamber longitudinal strain, GLS: global longitudinal strain, GCW: global constructive work, GWW: global wasted work, GWI: global work index, GWE: global work efficiency, IVSDd: intraventricular septum diameter measured in diastole, PWDd: posterior wall diameter measured in diastole, LVDd: left ventricular internal diameter measured in diastole, TR: tricuspid regurgitation, LA: left atrium, ANCOVA: analysis of covariance

greater in the male participants versus the female participants (1.1% (95% CI -0.3, 2.5) (p=0.12)).

Lower BW, but not GA was significantly associated with more impaired LV-GLS (PCC −0.2 with p=0.02).

Among the PB/ELBW, 4 had reduced EF Simpson, 11 had borderline impaired LV-GLS, and 3 had impaired LV-GLS. Among the controls one had reduced EF Simpson, 6 had borderline impaired LV-GLS, and none had impaired LV-GLS.

Diastolic LV function

Parameters of LV diastolic function were mainly normal and similar in the PB/ELBW and controls. Of the PB/ELBW, 2 had abnormally low septal e´ velocity, 3 had abnormally low lateral e´ velocity, none had abnormally high average E/e´, 1 had abnormally high tricuspid-valve regurgitation peak gradient, and none had abnormally high LA volume index.

Of the controls, 2 had abnormally low lateral e´ velocity, and 3 had abnormally high LA volume index, otherwise the parameters were normal. None of the participants had more than one abnormal parameter associated with diastolic dysfunction.

The mean LA reservoir strain, conduit strain, and contractile strain were similar in PB/ELBW and controls (Table 2; Fig. 5). Lower LA conduit strain, higher average E/e´, lower mitral septal e`, and lower mitral lateral e` were associated to increased chronological age (PCC −0.2 with p=0.02, PCC 0.3 with p=0.001, PCC −0.5 with p=<0.01, and PCC −0.2 with p=0.01, respectively), but not associated with BW or GA.

LA reservoir strain, conduit strain, and contractile strain were also similar in PB/ELBW and controls when re-analysed while using atrial cycle with zero reference point set at the start of the ECG p-wave.

Myocardial work MW was calculated after determination of LV-GLS and BP in 49 PB/ELBW and 51 controls. Mean LV-GLS was significantly impaired and mean BP was similar in these PB/ELBW compared to these controls. Mean LV-GLS was −19.4% (95% CI -20.0, -18.9) in PB/ELBW and −20.6 (95% CI -21.1, -20.1) (p=0.003) in the controls. Mean BP was 112/69 mmHg (SD 11/7) in PB/ELBW and 111/69 mmHg (SD 11/8) in controls (p=0.9 and 1.0). One control had systolic BP>140 mmHg, otherwise BP were below 140/90 mmHg in all participants.

MW is influenced by the contraction power of the myocardial fibres, the LV loading conditions, and the wall stress applied on the LV segments [37]. LV dimension must be taken into consideration since calculation of work would be relatively underestimated in dilated ventricles due to higher wall stress at any given LV pressure than in smaller ventricles [38]. The PB/ELBW and controls had seemingly similar loading conditions and wall

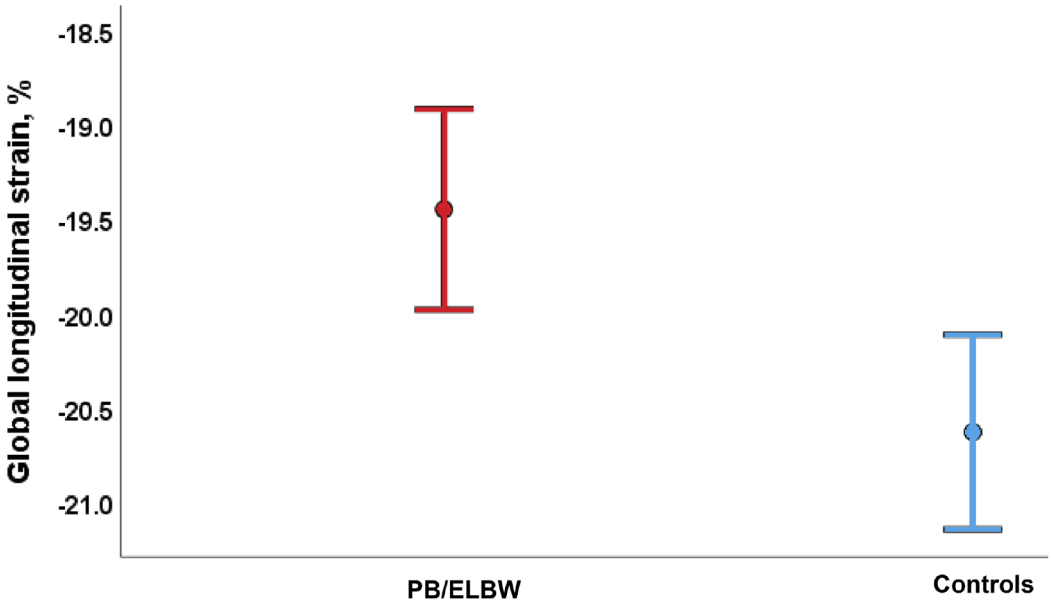

Fig. 4 Left ventricular myocardial function measured by peak global longitudinal strain (GLS) was significantly reduced in young adults born very premature or with extremely low birthweight (PB/ELBW) compared to controls. Mean GLS was −19.4% (95% confidence interval (CI) -20.0, -18.9) in PB/ELBW and −20.6% (95% CI -21.1, −20.1) in controls (p = 0.003)

stress due to similar means of parameters describing diastolic function and LV filling pressure, BP, heart rate, LV dimension measured by end-diastole internal diameter, and myocardial mass index (Tables 1 and 2).

The MW described as means of GWI, GCW, GWW, and GWE were not significantly different in PB/ELBW and controls, and the measures were close to former described reference values [21]. However, there was a trend towards lower GWI and GCW and higher GWW in PB/ELBW compared to controls (Table 2; Fig. 6).

GWI, GCW, GWW, and GWE were not significantly associated to BW or GA.

Participant characteristics and echocardiographic findings across the three cohorts of PB/ELBW are described in a table in Additional file 2. There were no differences between the cohorts regarding measures of left ventricular systolic and diastolic function, including measures of MW. Additionally, there was no significant associations between treatment in the prenatal and neonatal period (days of oxygen supplement, days of ventilation support, use of surfactant, or steroid treatment) and measures of myocardial function.

Discussion

In 63 young adults born very preterm or with extremely low BW, we found impaired LV-GLS compared to sex- and age matched controls, but mainly normal systolic function including estimates of MW. Diastolic function including measures of LA longitudinal strain was preserved. Lower BW was associated with more impaired LV-GLS and increasing chronological age was associated with decreasing diastolic function.

Our finding of similar EF in young adults born preterm or with low BW compared to controls, but reduced LV function measured by longitudinal strain, are similar to studies by Huckstep et al. [13] and Lewandowski et al.[14, 15]. However, Goss et al. [18] reported hypercontractile LV function measured by longitudinal strain in 38 young adults at mean age 25 years, born at mean GA 29 weeks. While the studies by Huckstep and Levandowski contained preterm born participants with significantly higher systolic BP and/or higher myocardial mass [13–15], the preterm participants in the study by Goss had lower myocardial mass and similar systolic BP compared to their controls [18]. This may contribute to the difference in result since the study participants otherwise were seemingly similar. Increased LV mass has independently been associated with more impaired LV-GLS in other groups, such as patients with aortic stenosis [39, 40] and athletes [41, 42]. Additionally, strain measurements are known to be afterload dependent with more impaired longitudinal strain with higher BP [32, 38]. Low BW has been classified by the World Health Organization [43] as a risk factor for cardiovascular disease. This corresponds

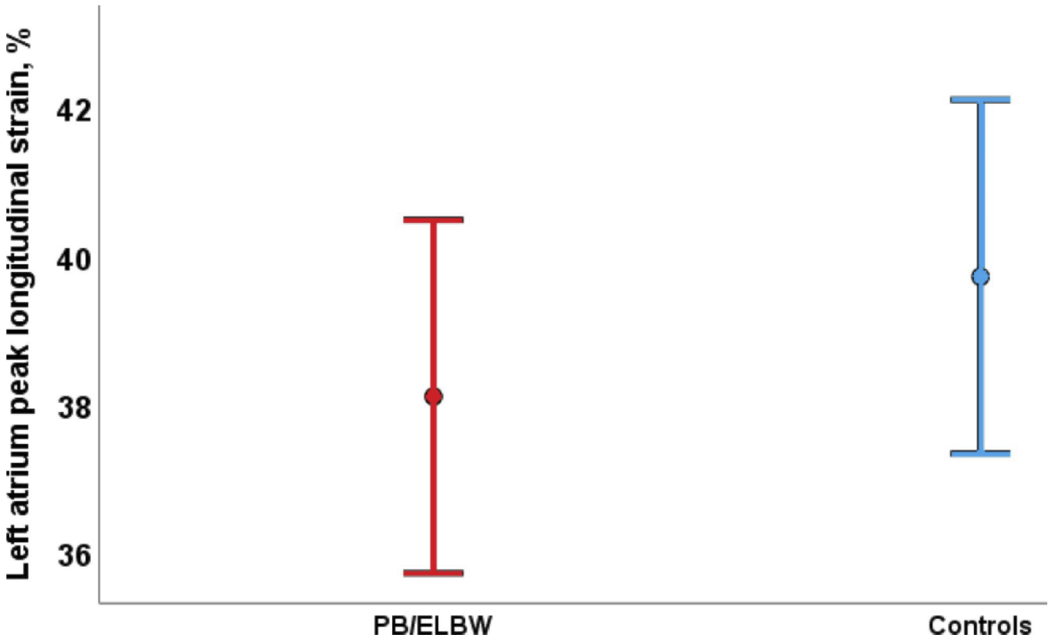

Fig. 5 Left atrial reservoir function measured by peak longitudinal strain was similar in young adults born very preterm or with extremely low birthweight (PB/ELBW) and controls. Mean left atrial reservoir strain was 38.1% (95% confidence interval (CI) 35.7, 40.5) in PB/ELBW and 39.7% (95% CI 37.4, 42.1) in controls (p = 0.3)

well to our finding of lower BW being associated to more impaired LV-GLS. Although morbidity and all-cause mortality of young adults (15- <50 years) born preterm are reported higher in men compared to women [2], the difference in LV-GLS between PB/ELBW and controls in our study did not differ significantly between sexes.

Similar to our study, Goss et al. [18] reported preserved diastolic function in preterm born young adults. However, several previous studies have found reduced diastolic function in this group. A meta-analysis from Telles et al. [44] and a study by Lewandowski et al.[15] reported an overall lower LV diastolic function in preterm born children and young adulthood as well as indications of increased diffuse myocardial fibrosis. Recently, analysis of LA reservoir strain has been reported to detect increased LV filling pressure and LV diastolic dysfunction at an earlier point than other echocardiographic measures [24, 25]. We found no significant difference between mean LA reservoir strain in PB/ELBW and controls, and a mean LA reservoir strain in PB/ELBW of 38.1% which is close to a formerly described mean reference value (39%) in healthy subjects [36]. These findings support a normal diastolic function in our preterm born young adults. Diverging results between studies may reflect the complex interplay of altered cardiac structure and loading conditions on measures of diastolic function. Aging is associated with reduced LV relaxation, which may lead to diastolic dysfunction [31]. This corresponds well with our finding of a significant association between increased chronological age and lower diastolic function measured by LA conduit strain, E/e´, and mitral septal and lateral e´.

To add further information on myocardial function we estimated MW non-invasively. In subjects with cardiac remodelling and elements of myocardial fibrosis, which has been reported in preterm born young adults [15, 44], we might expect reduced myocardial function with time which can be expressed by decreased GWI and GCW, and increased GWW. Similar to reported findings in subjects with hypertrophic cardiomyopathy [45, 46], we found a trend towards reduced GWI and GCW, and higher GWW, in the PB/ELBW group compared to the controls, although not significantly different.

The additive value of these new parameters describing myocardial function over the traditional ones is still object of study, and further studies are needed to establish clinical utility and prognostic impact. Estimation of MW could add information to distinguish whether reduced contraction patterns are caused by newly emerged hypertension or attenuated contractility, since

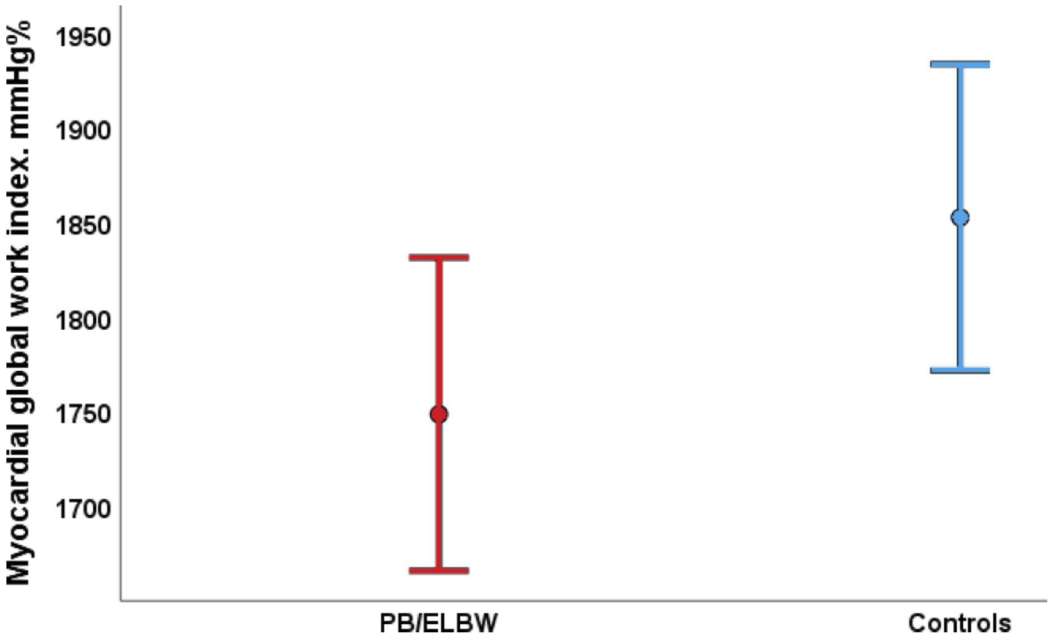

Fig. 6 Myocardial performance measured by global work index (GWI) generated from left ventricle pressure-strain loops was similar in young adults born very preterm or with extremely low birthweight (PB/ELBW) and controls. Mean GWI was 1749 mmHg% (95% confidence interval (CI) 1666, 1832) in PB/ELBW and 1853 mmHg% (95% CI 1772, 1934) in controls (p = 0.08)

the increased afterload will reduce EF and LV-GLS, but increase GWI and GCW [38]. This may be especially important in preterm born adults, who have increased prevalence of hypertension [47].

Our study population consisted of three cohorts born in different decades (early 1980s to 2000), in a period with significant changes in the neonatal medical treatment. There was no difference in parameters of left ventricle myocardial function across the cohorts. However, the study samples within the cohorts of the preterm born were too small to reveal minor group differences regarding myocardial function.

There has been an increasing use of prenatal corticosteroids and assisted ventilation during the period, and the use of surfactant was introduced in the early 1990s. Additionally, the techniques have improved, from assisted ventilation mainly by intermittent or continuous positive pressure ventilation via an endotracheal tube in the early period to increasing use of nasal continuous airway pressure and high frequency oscillatory ventilation during the 1990s [48]. The PB/ELBW participants born in 1999–2000 (cohort 3) had significantly lower mean birthweight compared to those born in 1982–1985 (cohort 1) which may indicate increasing survival of the most vulnerable neonates. While the lower mean birthweight in the youngest cohort compared to the oldest cohort (857 g versus 1014 g) could have led to a decreased left ventricular myocardial function in this subgroup, the possible improvement in neonatal intensive care treatment the last decades, and the younger age at the time of assessment, could have the opposite influence on myocardial function in the younger cohorts. To further investigate this, we correlated measures of myocardial function to the treatment in the prenatal and neonatal period (days of oxygen supplement, days of ventilation support, use of surfactant, or steroid treatment), and found no significant associations. Additionally, the measures of myocardial function were similar in the PB/ELBW with moderate to severe bronchopulmonary dysplasia (n = 33) compared to the participants without this diagnosis, and in the PB/ELBW with extremely low BW (n = 37) or extremely preterm birth (n = 39) compared to the remaining PB/ELBW.

Strengths and limitations

This study is part of a long-term cohort study following individuals born PB/ELBW and individually age and sex matched term-born controls. The study design, and similarity of the PB/ELBW and control group at the time of the assessment reduces the risk of confounding. To diminish procedure related variance the echocardiographic imaging and off-line analyses were performed by one highly trained sonographer using the same

Table 3 Characteristics of participants and non-participants born very preterm or with extremely low birthweight

	Participants (n = 63)	Non-participants (n = 85)	p
Male, n	27 (43%)	47 (55%)	
Cohort 1 born 1982-85, n (%)	23 (37%)	26 (31%)	
Cohort 2 born 1991-92, n (%)	19 (30%)	16 (19%)	
Cohort 3 born 1999–2020, n (%)	21 (33%)	43 (51%)	
BW, grams, mean (SD)	948 (216)	896 (179)	0.1[a]
GA, weeks, mean (SD)	27 (2)	27 (2)	1.0[b]
Days on ventilator, mean (SD)	8 (9)	8 (11)	0.4[b]
Days on CPAP, mean (SD)	33 (14)	29 (20)	0.3[a]
Days on O_2-supplement, mean (SD)	52 (35)	57 (45)	0.4[b]
Maternal smoking, n (%)	21 (34%) (n = 62)	29 (37%) (n = 77)	1.0[b]
Moderat/severe BPD, n (%)	21 (33%)	37 (44%)	0.8[b]
Pre-natal steroid treatment, n (%)	35 (55%)	48 (56%)	1.0[b]
Post-natal steroid treatment, n (%)	14 (22%)	20 (24%)	1.0[b]
Surfactant, n (%)	28 (44%)	43 (50%)	1.0[b]

[a] = independent sample t test (with variance not assumed)

[b] = non–parametric two sample Kolmogorov– Smirnov test

BW: birthweight; SD: standard deviation; GA: gestational age; CPAP: continuous positive airway pressure; O_2: oxygen; BPD: bronchopulmonary dysplasia

ultrasound system and echocardiographic software. The calculated intra- and inter-rater variability were good to excellent [49].

There were limitations to the study. Only 42% of the eligible PB/ELBW subjects were examined. Those who did not participate had similar GA, occurrence of moderate or severe bronchopulmonary dysplasia [50], and days of ventilatory support and oxygen demand, compared to those who did participate (Table 3). The non-participants had a slight and non-significant lower mean BW compared to the participants (896 g versus 948 g, p = 0.1). A possible selection bias could therefore most likely have led to an underestimation of the difference in echocardiographic measures between PB/ELBW and controls since lower BW was associated with more impaired myocardial function.

Only four chamber view images were used for analyses of LA strain while reference values and guidelines are based on measures of two- and four chamber view images. The mean values for LA strain might therefore deviate from reference values, but would not affect the analysis of group difference between PB/ELBW and controls. However, the mean LA peak longitudinal strain in our control group (39.7%) was similar to former reported reference values in healthy subjects (39%). Although R-R gated LA strain analysis is recommended in the guidelines and more frequently used, P-P gating has been found to correlate better to new-onset heart failure and to 3-dimentional echocardiographic-derived measures [51, 52]. LA strain was re-analysed using P-P gating with zero reference point set at the start of the ECG P-wave. As expected, this generated lower LA peak longitudinal strain values (reservoir strain) [36], but did not change the main result when mean LA strain values in PB/ELBW and control were compared.

LV-GLS, but not estimates of MW, were found impaired in PB/ELBW compared to controls. The study was not designed to prove the superiority of MW to LV-GLS, and although analyses of MW have been considered an advancement of LV strain analyses due to reduction of the strain analysis` load dependent limitations [20, 21], the method cannot be considered load-independent, as it is derived from strain measurements. Larger scale studies are needed to establish the clinical utility and prognostic impact of MW on cardiovascular outcome in preterm born young adults.

Conclusion

Young adults born very preterm (< 29 weeks of gestation) or with extremely low BW (< 1000 g) had impaired LV-GLS compared to term-born controls, although mainly normal systolic function. Myocardial function measured by MW and diastolic function were preserved. Lower BW was associated with more impaired LV-GLS and increased chronological age was associated with decreasing diastolic function. These findings could indicate an elevated risk in preterm born young adults of developing heart failure. Given the increasing survival rate of preterm born neonates it is of public health interest to generate prevention strategies to reduce modifiable risk factors and to implement long-term cardiovascular follow-up in this group. Further studies are needed to investigate the impact of preterm birth and low BW on myocardial function in young adulthood to optimize risk stratification.

Abbreviations

AFI	automated function imaging
ANCOVA	analyses of covariance
BP	blood pressure
BPD	bronchopulmonary dysplasia
BSA	body surface area
BW	birthweight
CL	confidence interval
CPAP	continuous positive airway pressure
EF	ejection fraction
GA	gestational age
GCW	global constructive work
GLS	global longitudinal strain
GWE	global work efficiency
GWI	global work index
GWW	global wasted work
LA	left atrial
LV	left ventricle
LV-4C-LS	left ventricle longitudinal strain from the apical four chamber view
MW	myocardial work
O_2	oxygen
PCC	Pearson correlation coefficient
PB/ELBW	very preterm born or with extremely low birthweight

ROI region of interest
SD standard deviation

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Supplementary Information

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Additional File 1: Flow chart of the study population

Additional File 2: Characteristics and echocardiographic measures of participants born very preterm/with extremely low birthweight by cohort

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Author contributions
Britt Engan, Tom R. Omdal, Gottfried Greve, Elisabeth Leirgul and Maria Vollsæter contributed to the study conception and design. Material preparation, data collection and analysis were performed by Britt Engan. All authors participated in the interpretation of the data, and critically reviewed and commented on the first and previous drafts of the manuscript written by Britt Engan. All authors approved of the final manuscript.

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Data availability
In accordance with the approvals granted for this study by The Regional Committee on Medical Research Ethics and The Norwegian Data Protection Authority, the data files are stored securely and in accordance with the Norwegian Law of Privacy Protection. The data file cannot be made publicly available as this might compromise the respondents' privacy. Some of the participating centres are small and the number of extremely preterm births limited with a risk of identifying anonymous participants. To prepare future research papers other researchers in our group currently use the data file. A subset of the data file with anonymized data can be made available to interested researchers upon reasonable request to Maria Vollsæter (maria.vollseter@helse-bergen.no), providing Norwegian privacy legislation and GDPR are respected, and that permission is granted from The Norwegian Data Protection Authority and the data protection officer at Haukeland University Hospital.

Declarations

Competing interests
The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Ethics approval and consent to participate
The study was carried out in accordance with the Helsinki declaration and were approved by the Regional Committee for Medical and Health Research Ethics of the Western Norway Health Authority (REC 2017/0068). Written informed consent was obtained from all participants.

Consent for publication
Not applicable.

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Publisher's Note

Systolic myocardial function measured by echocardiographic speckle-tracking and peak oxygen consumption in pediatric childhood cancer survivors - a PACCS study

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Keywords: pediatric childhood cancer survivors, myocardial function, speckle-tracking echocardiography, post-systolic shortening, peak oxygen consumption.

Abstract

Background

Cancer therapy related cardiotoxicity is a major cause of cardiovascular morbidity in childhood cancer survivors. The aims of this study were to investigate systolic myocardial function and its association to cardiorespiratory fitness in pediatric childhood cancer survivors.

Methods

In this sub-study of the international study "Physical Activity and fitness in Childhood Cancer Survivors" (PACCS), echocardiographic measures of left and right ventricular global longitudinal strain (LV-GLS and RV-LS) were measured in 128 childhood cancer survivors aged 9 to 18 years and in 23 age and sex matched controls. Cardiorespiratory fitness was measured as peak oxygen consumption achieved on treadmill and correlated to myocardial function.

Results

Mean LV-GLS was reduced in the childhood cancer survivors compared to the controls, -19.7 % (95% confidence interval (CI) -20.1, -19.3) versus -21.3% (95% CI -22.2, -20.3) (p= 0.004). Mean RV-LS was similar in the childhood cancer survivors and the controls, -23.2% (95% CI -23.7, -22.6) versus -23.3% (95% CI -24.6, -22.0) (p= 0.8). In the childhood cancer survivors, lower myocardial function was associated with lower peak oxygen consumption (correlation coefficient (r)= -0.3 for LV-GLS). Higher doses of anthracyclines (r= 0.5 for LV-GLS and 0.2 for RV-LS) and increasing time after treatment (r= 0.3 for LV-GLS and 0.2 for RV-LS) were associated with lower myocardial function.

Conclusions

Left ventricular function, but not right ventricular function, was reduced in pediatric childhood cancer survivors compared to controls. A lower left ventricular myocardial function was associated with lower peak oxygen consumption suggestive of clinically significant reduction of left ventricular function. Further, higher anthracycline doses and increasing time after treatment were associated with lower myocardial function, implying that long-term follow-up is important in this population at risk.

Introduction

Cardiovascular disease is a leading non-cancer contributor to early morbidity and mortality in childhood cancer survivors (CCSs) (1). In addition to the poorer cardiovascular risk profile observed in the CCSs compared to the general population (1), exercise intolerance and decreased maximal oxygen consumption, associated with increased all-cause mortality, are reported to be prevalent among adult CCSs (2). Exercise performance is also reported reduced in a few small studies of pediatric CCS populations (3-5).

Cancer treatment includes a variety of chemotherapeutic medication and radiation therapy, associated with dose-dependent cardiovascular toxicity (6). Younger age at the time of treatment and female sex have been associated with increasing treatment-related cardiotoxicity (1). However, not only cancer treatment, but also the disease itself might be a risk factor for cardiovascular late effects, due to chronic inflammation and oxidative stress present in all cancer diseases (7).

Despite the development of modern targeted therapies, anthracycline chemotherapy is used to treat a wide spectrum of childhood cancers (8). Anthracyclines belong to the class of chemotherapeutic drugs with most prominent association with cardiotoxicity (1), and are known to cause acute and late cardiomyopathy, often irreversible and progressive (6). Other classes of antineoplastic drugs, such as vinca alkaloids and platinum derivates, have mainly been linked to cardiac ischemia, thromboembolism, and hypertension, but also heart failure (9).

Echocardiography continues to be the mainstay in the evaluation of myocardial function related to cancer treatment. Compared to traditional measures of myocardial function such as ejection fraction (EF) and fractional shortening, measures of speckle-tracking derived strain have shown to be more sensitive to changes in left ventricular myocardial function, and to provide additional prognostic information (10, 11). Former studies of myocardial function in CCSs measured by echocardiographic strain have shown more global than regional impairment of left ventricular function, and impairment of the right ventricle, suggesting a uniform effect of cancer and its treatments on the myocardium (11-13). Echocardiographic speckle-tracking allows measures of post-systolic shortening (PSS) which represents delayed myocardial contraction, in terms of longitudinal shortening, occurring after end-systole. PSS has been described in myocardial segments with contractile dysfunction due to ischemia and hypertrophy and is associated with increased risk of cardiovascular events in high-risk populations (14-16). PSS has also been observed in the myocardium of healthy and low risk subjects, and even in this population reported to be a predictor of cardiovascular morbidity and death (17, 18).

Peak oxygen consumption (VO_2) is mainly limited by cardiac output (19), and reduced stroke volume is correlated to reduced myocardial function measured by strain (20). Myocardial function measured by strain, and cardiorespiratory fitness have to date mainly been investigated in adult survivors of childhood cancer. However, evaluation of cardiac function and fitness in pediatric CCSs is of importance to identify cardiovascular risk at an early point. The aim of the present study was to investigate biventricular myocardial function by echocardiographic measures of left and right ventricular longitudinal strain and PSS, and the association between myocardial function and cardiorespiratory fitness measured by peak VO_2, in pediatric CCSs. Our hypothesis was that myocardial function is reduced in pediatric CCSs compared to controls and associated with lower cardiorespiratory fitness.

Materials and methods

Study design and participants

This study was a sub-study of the international multicenter cohort study "Physical Activity and fitness in Childhood Cancer Survivors" (PACCS) (21) with main aims to identify level of and mediators of activity and physical fitness in CCSs. Children and adolescents aged 9 to 18 years with childhood cancer treatment finished at least one year before, were invited to participate in the study when attending routine follow-up at oncological outpatient clinics at their hospitals. Exclusion criteria were severe activity triggered arrhythmia, heart failure with echocardiographic measured fraction shortening (FS) below 20%, or physical function too poor to enable the completion of the physical tests. Healthy age- and sex matched schoolmates were recruited as control participants.

In the present sub-study, CCSs from the pediatric cancer outpatient clinic at Oslo University Hospital (Oslo, Norway) and Haukeland University Hospital (Bergen, Norway) were invited to participate. Of the 207 eligible CCSs from the PACCS study, 66 declined the invitation and 13 withdrew after inclusion. No one was excluded due to heart failure, arrhythmia, or too low physical capacity. A total of 128 CCSs underwent echocardiographic examination, and 126 underwent cardiopulmonary exercise test. Due to capacity challenges regarding echocardiographic imaging only one center, Haukeland University Hospital, recruited controls, and 50 controls were invited. The participants were examined in the period from February 2019 to February 2021. Due to the ongoing COVID-19 pandemic with several restrictions regarding travelling and entrance to the hospitals, and frequent COVID-quarantine in school children, only 23 controls were included.
The CCSs had been treated for many different cancer forms and were categorized according to the international classification of childhood cancer, third edition (ICCC3) (22). The cumulative doses of anthracyclines were calculated as equipotent doses of doxorubicin (mg/m^2) according to Children´s Oncology Group´s long-term follow-up guidelines version 5.0 (23) and Feijen et al (24). (Table 1).

Table 1
Equipotent doses of doxorubicin

Doxorubicin	Multiply total dose x 1
Daunorubicin	Multiply total dose x 0.5
Epirubicin	Multiply total dose x 0.67
Idarubicin	Multiply total dose x 5
Mitoxantrone	Multiply total dose x 4

Cumulative equipotent doses of doxorubicin (mg/m^2) calculated according to Children´s Oncology group long-term follow-up guidelines version 5.0 (http://www.survivorshipguidelines.org/)

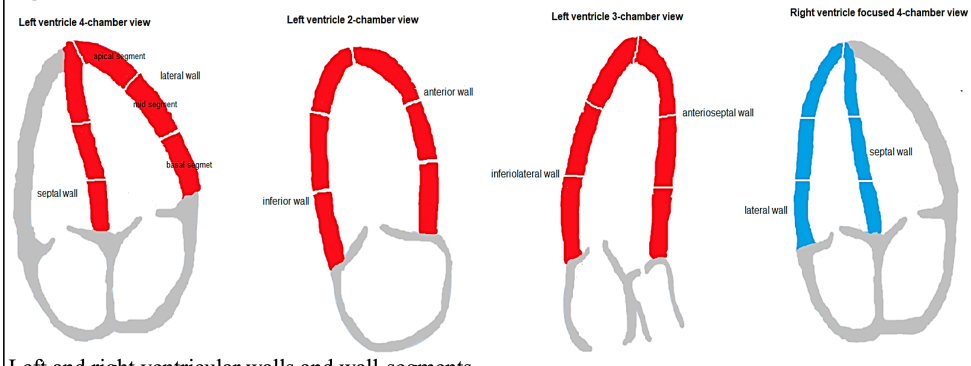
The study was conducted in accordance with the Helsinki declaration.
The Regional Committee for Medical and Health Research Ethics of the South-East Norway Health Authority approved the study (REC 2018/739). Written and verbal information were given to the participants adapted to their age and developmental stage. Written informed consent was obtained from all participants and their parents or legal guardians.

Echocardiographic assessment

All echocardiographic examinations were performed with Vivid E9 ultrasound system (GE Healthcare, Horten, Norway) using a 4Vc-D (1.4-5.2 MHz) transducer for all imaging in Bergen and an equivalent 5Sc (1.5-4.6 MHz) transducer for all imaging in Oslo. Data was stored on external hard drives or Digital Versatile Discs allowing offline analyses with the echocardiographic software, EchoPAC version 204 (GE Healthcare, Horten, Norway). The participants were examined at rest in supine position. No sedation was used. All the participants underwent a comprehensive functional echocardiographic examination including grayscale images optimized for 2-dimensional (2D) speckle analysis. Quantification of systolic left ventricular (LV) and right ventricular (RV) function was performed following the recommendations from the European and American societies of echocardiography (25, 26).

LV-EF was calculated by the modified Simpson biplane technique. Gray scale images for 2D speckle-tracking analysis were acquired at frame rate (frames/second) to heart rate (beats/minute) ratio 0.7-0.9. Longitudinal strain curves from standard apical four, three, and two chamber view images, analyzed for 18 sub-segments, were used for LV longitudinal strain assessments. Longitudinal strain curves from a RV-focused four chamber view image, analyzed for 6 sub-segments including the interventricular septum, were used for RV longitudinal strain assessments (figure 1).

Figure 1



Left and right ventricular walls and wall-segments.

A single cardiac cycle considered to have the most optimal images was selected for analysis of LV and RV 2D strain (27, 28). The region of interest (ROI) was defined by manual tracing along the endocardial border from the atrioventricular valve annulus to the apex, and the thickness adjusted to cover the myocardium without including the pericardium. Tracking was performed automatically. If necessary, the ROI was adjusted manually, until the best possible tracking was obtained. Tracking compromised by artefacts/shadows, or image acquisitions with more than one faulty segment were excluded. Peak systolic strain was measured at aortic valve closure for LV measurements and at pulmonary valve closure for RV measurements. The timing of the aortic and pulmonary valve closure was assessed by continuous-wave Doppler.

In this paper, LV global longitudinal strain (LV-GLS) is used to describe the average peak systolic longitudinal strain from all three left ventricular apical views, and the term RV longitudinal strain (RV-LS) is used to describe the peak systolic RV longitudinal strain from a RV-focused four chamber view, including RV free wall and intraventricular septum. All longitudinal strain values are described as negative percentages, as it describes myocardial shortening of segments relative to end-diastolic length.

LV longitudinal strain (LV-LS) z-scores were calculated as proposed by Dallaire et al. (29) from measurements in apical four chamber view images, normalized to body surface area (BSA) and adjusted for heteroscedasticity. The z score calculation was based on imaging and analyses with similar echocardiographic ultrasound system and off-line software as in used in our study (29) and obtained from the equation (with BSA calculated by using the equation proposed by Haycock et al (30)):

$$Z = \frac{(\text{observed value} - 20.295 \times \text{BSA}^{0.5})}{(-0.343 \times \text{BSA} + 2.02)}$$

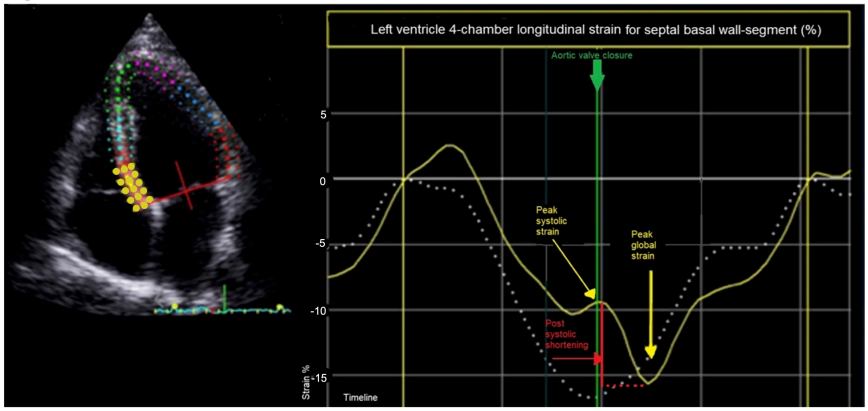
LV-LS z-score < -2 was considered abnormally low.

Post systolic shortening (PSS), defined as late systolic longitudinal shortening appearing after the aortic valve closure in one cardiac cycle, was derived from calculations of post systolic index (PSI). PSI was defined as longitudinal shortening after aortic closure relative to maximal global longitudinal strain

$$\text{PSI} = \frac{(\text{peak global strain} - \text{peak systolic strain})}{(\text{peak global strain})} \times 100\%$$

If maximal global longitudinal strain was within the systole, PSI was set to zero. PSS was defined as PSI > 0% (figure 2).
For analysis, the average PSI of basal, mid, and apical LV wall-segments, the average PSI of septal, lateral, anterior, inferior, anteroseptal, and inferolateral LV walls, and the average PSI of all LV and RV segments were used (figure 1). The terms global LV PSI and global RV PSI were used to describe the average PSI of all LV and RV segments. Additionally, we registered the number of LV and RV wall-segments with any degree of PSS.

Figure 2



Speckle-tracking derived longitudinal strain of basal septal wall-segment (yellow line) in left ventricle 4-chamber image. Post systolic shortening (red line) is defined as late systolic longitudinal shortening appearing after the aortic valve closure (green line) in one cardiac cycle and is calculated as post systolic index:

$$\text{PSI} = \frac{(\text{peak global strain} - \text{peak systolic strain})}{(\text{peak global strain})} \text{ x } 100\%$$

In this example:

$$\text{PSI} = \frac{(-15\%) - (-10\%)}{(-15\%)} \text{ x } 100\% = 33\%$$

The dashed white line represents longitudinal strain of a wall-segment without post systolic shortening.

All analyses were performed by a single experienced cardiologist (B.E). Randomly selected study participants (5%) were obtained for inter-rater and intra-rater analyses. Another experienced cardiologist (T.O) performed the re-analyses for inter-rater reliability. Both were blinded to the original results during re-analysis. Inter-rater reliability measured by a 2-way mixed-effect model for absolute agreement was 0.8 (95% confidence interval (CI) 0.1, 1.0) for EF Simpson, 0.9 (95% CI 0.4, 1.0) for LV-GLS, and 0.9 (95% CI 0.6, 1.0) for RV-LS. Intra-rater reliability measured by a 2-way mixed-effect model for absolute agreement was 0.7 (95% CI -0.3, 0.9) for EF Simpson, 0.9 (95% CI 0.5, 1.0) for LV-GLS, and 0.9 (95% CI 0.6, 1.0) for RV-LS.

Cardiopulmonary exercise test

Cardiopulmonary exercise test was performed by walking/running on a treadmill (Woodway PPS MED, WOODWAY USA, Waukesha, WI, USA) with an incremental ramp protocol until exhaustion. The participant was breathing into a Hans Rudolph two way breathing mask (2700 series; Hans Rudolph Inc, Kansas City, USA) connected to an OxyconPro analyzer (Jaeger, Würtzburg, Germany) using the breath-by-breath method. Borg scale (scale 6-20) (31) was used as rating of perceived exertion immediately after the test. The highest oxygen uptake measured, which remained stable over at least 30 seconds, was used as peak oxygen consumption (peak VO_2) if two of the following three criteria were fulfilled: respiratory gas exchange ratio ≥1.10; Borg scale ≥17; or VO_2 reached a plateau with increasing workload. The result for the CCSs were included in the present study, to correlate peak VO_2 with measures of myocardial systolic function.

Anthropometry and blood pressure

Weight and standing height were measured with the participants wearing light clothing. Resting blood pressure was measured with an automated oscillometric device (in Bergen, Biolight BLT V6, Biolight Meditech Company, China, and in Oslo, Connex ProBP 3400, Welch Allyn, USA) after 5 minutes of rest in supine position, using the lowest of two measurements.

Blood samples

Venous blood samples were collected from the CCSs only, in serum tubes and Ethylene-Diamine-Tetra-acetic Acid containing tubes, for measurements of Hemoglobin (Hb) and N-terminal pro-Brain Natriuretic peptide (NT-proBNP). NT-proBNP values > 160 ng/L was defined as above the upper limit in children aged 6-18 years (32).

Statistical analyses

Descriptive variables and outcome data are presented as means with standard deviation (SD) or 95% CI. For comparison of descriptive variables and outcome data (without significant confounders) between CCSs and controls, independent samples t-tests (with equal variance not assumed), two samples Kolmogorov-Smirnov test, and Chi-square test or Fisher`s exact test were applied as appropriate.

Linear regression analysis was used to identify confounders significantly affecting the analyses. Analyses of covariance (ANCOVA) were used to compare CCSs and controls for height, BSA, and BMI adjusted for sex; blood pressure adjusted for sex and age; EF and LS z-score adjusted for echocardiographic transducer difference; longitudinal strain adjusted for transducer difference and BSA; and peak VO_2 adjusted for sex, age, and treadmill location.

All comparisons were re-analyzed without outliers, which did not significantly affect the results.

To examine whether the differences in EF, LV-GLS, and RV-LS between the CCSs and controls differed by sex an interaction term for sex and group affiliation was added.

Comparisons of descriptive variables and outcome data between subgroups of study participants were done by Chi-square test or Fisher`s exact test, Welch´s ANOVA or classic one-way ANOVA (with Games-Howell and Tukey`s test used for post hoc analyses, respectively), or by multiple comparison analyses (with Sidak correction) with the same adjustments as described above, as appropriate.

Pearson correlation coefficient (PCC) was used to explore associations between systolic myocardial function and treatment exposure, peak VO_2 (adjusted for transducer/treadmill, sex, and age), age at diagnosis, and time after treatment, and between NT-ProBNP and anthracycline dose or peak VO_2.

All tests were two-sided, and $p < 0.05$ was considered statistically significant. All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY, USA).

Results

Participant characteristics

Characteristics of the 151 study participants; 128 CCSs and 23 controls, are described in table 2. The mean age was higher in the CCSs than in the control group, 13.6 years (SD 2.6) and 12.7 years (SD 3.1), respectively ($p = 0.04$), and mean systolic BP was lower in the CCSs compared to the controls, 107 mmHg versus 112 mmHg, respectively ($p = 0.03$). The CCSs and controls were similar regarding sex and ethnicity distribution, and estimated means of height, weight, BSA, BMI, and diastolic blood pressure.

For the CCSs, the mean age at diagnosis was 5.3 years (SD 0.3), and mean time after treatment was 6.6 years (SD 3.6). The most frequent diagnosis in the cancer survivor group was acute leukemia (48%), cerebrospinal neoplasms (13%), and renal tumors (11%). Most participants received multiple chemotherapeutic agents during the treatment period, detailed in table 2. The majority, 78%, was treated with anthracyclines, with mean dose 152 mg/m^2 (SD 91), while 22 % were anthracycline naive and treated with vinca alkaloids and/or platinum derivates. Six (5%) CCSs underwent radiation therapy involving the chest, all of these had also received chemotherapy. Four (3%) had surgical treatment, but no chemo- or radiation therapy (all diagnosed with cerebrospinal neoplasms).

Four CCSs had NT-proBNP values > 160 ng/L defined as the upper limit in children aged 6-18 years (32), and mean NT-proBNP was 67 ng/L (SD 48).

Table 2
Characteristics of pediatric childhood cancer survivors and controls

	CCS (n = 128)		Controls (n = 23)		p
	n		n		
Age at study, years, mean (SD)		13.6 (2.6)		12.7 (3.1)	0.04[a]
Sex, males	66	52%	10	44%	0.48[b]
Ethnicity, Caucasian	117	91%	23	100%	0.79[b]
Ethnicity, Asian	3	2%			
Ethnicity, mixed	7	6%			
Ethnicity, other	1	1%			
Height, cm, mean (95% CI)		158 (155, 160)		153 (147, 158)	0.11[c]
Weight, kg, mean (SD)		51 (15)		45 (14)	0.08[d]
BMI, kg/m^2, mean (95% CI)		20.2 (19.6, 20.9)		19.0 (17.5, 20.5)	0.14[c]
BSA, m^2, mean (95% CI)		1.5 (1.4, 1.5)		1.4 (1.3, 1.5)	0.09[c]
Systolic BP, mmHg, mean (95% CI)		107 (105, 108)		112 (108, 116)	0.03[c]
Diastolic BP, mmHg, mean (95% CI)		65 (64, 67)		69 (65, 73)	0.07[c]
Age at diagnosis, years, mean (SD)		5.3 (3.5)			
Time since diagnosis, years, mean (SD)		8.3 (3.7)			
Time since last treatment, years, mean (SD)		6.6 (3.6)			
ICCC3-1 (leukemias)	62	48%			
ICCC3-2 (lymphomas)	12	9%			
ICCC3-3 (CNS neoplasms)	17	13%			
ICCC3-4 (PNS tumors)	8	6%			
ICCC3-5 (retinoblastoma)	2	2%			
ICCC3-6 (renal tumors)	14	11%			
ICCC3-7 (hepatic tumors)	1	1%			
ICCC3-8 (malignant bone tumors)	6	5%			
ICCC3-9 (extraosseous sarcomas)	6	5%			
Chest radiation, Gy, mean (SD)	6	20 (9)			
Chemotherapy	124	97%			
Anthracyclines	97	78%			
Anthracycline dose, mg/m^2, mean (SD)	95	152 (91)			
Anthracycline dose <100 mg/m^2, mg/m^2, mean (SD)	36	79 (5.8)			
Anthracycline dose 100-250 mg/m^2, mg/m^2, mean (SD)	44	152 (38)			
Anthracycline dose 250-500 mg/m^2, mg/m^2, mean (SD)	15	329 (63)			
Vinca alkaloids	115	93%			
Vincristine dose, mg/m^2, mean (SD)	110	28 (13)			
Vinblastine dose, mg/m^2, mean (SD)	3	144 (148)			
Vinorelbine dose, mg/m^2, mean (SD)	1	706			
Platinum derivates	28	23%			
Cisplatin dose, mg/m^2, mean (SD)	13	289 (142)			
Carboplatin dose, mg/m^2, mean (SD)	24	3639 (294)			
NT-proBNP, ng/L, mean (SD)		67 (48)			
Hb, g/dL, mean (SD)		13.5 (1.0)			

a= non-parametric two sample Kolmogorov- Smirnov test

b= chi-squared test or fisher exact test

c = ANCOVA adjusted for sex and age as appropriate

d = independent samples t-test (with equal variance not assumed)

CCS: childhood cancer survivor, SD: standard deviation, CI: confidence interval, BMI: body mass index, BSA: body surface area, BP: blood pressure, ICCC3: international classification of childhood cancer-third edition, CNS: central nervous system, PNS: peripheral nervous system, NT-proBNP: N-terminal pro-Brain Natriuretic peptide, Hb: Hemoglobin, ANCOVA: analysis of covariance

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Left ventricle systolic function measured by EF

The CCSs had significantly lower mean EF Simpson compared to the controls (table 3). Mean EF Simpson was 60% (95% CI 59, 61) for CCSs and 64% (95% CI 61, 66) for controls (p = 0.01).

The difference in EF Simpson between CCSs and controls was not significantly greater in the female participants versus the male participants 2.7% (95% CI -1.7, 7.1) (p = 0.23).

Left and right ventricular systolic function measured by longitudinal strain

Due to poor acoustic conditions measurements of LV-GLS and RV-LS were excluded in 11 and 7 participants, respectively.

The CCSs had significantly reduced mean LV-GLS compared to the controls, while mean RV-LS did not differ significantly between CCSs and controls (table 3). Mean LV-GLS was -19.7% (95% CI -20.1, -19.3) for CCSs and -21.3% (95% CI -22.2, -20.3) for controls (p = 0.004) (figure 3) and mean RV-LS was -23.2% (95% CI -23.7, -22.6) for CCSs and -23.3% (95% CI -24.6, -22.0) for controls (p = 0.8)

The difference in LV-GLS and RV-LS between CCSs and controls was not significantly greater in the female participants versus the male participants, -0.3% (95% CI -2.1, 1.5) (p = 0.74) and 2.1% (95% CI -0.6, 4.8) (p=0.12), respectively.

None of the controls, but 16 (13%) of the CCSs had abnormally low LV-LS z-score < -2, and mean LV-LS z-score was reduced in the CCSs compared to the controls (table 3 and figure 4). Mean LV-LS z-score was -0.3 (95% CI -0.6, -0.1) for CCSs and 0.9 (95% CI 0.2, 1.5) for controls (p = 0.001).

Increasing time after treatment was associated with lower LV and RV systolic function with PCC 0.3 for LV-GLS (p = 0.004) and 0.2 for RV-LS (p = 0.02). Adjustment for anthracycline dose did not alter the result. We found no significant association between age at diagnosis, NT-proBNP, and the measurements of LV and RV systolic function.

Tabell 3
Measures of myocardial systolic function and peak oxygen consumption in childhood cancer survivors and controls.

	CCS		Controls		p
	n	**mean (95% CI)**	**n**	**mean (95% CI)**	
EF Simpson, %	128	60 (59, 61)	23	63 (61, 66)	0.01[a]
LV-4C-LS, %	126	-19.3 (-19.7, -19.0)	23	-21.2 (-22.1, -20.2)	<0.001[b]
Z-score LV-LS	126	-0.3 (-0.6, -0.1)	23	0.9 (0.2, 1.5)	0.001[a]
LV-3C-LS, %	118	-19.6 (-20.0, -19.1)	22	-20.5 (-21.7, -19.4)	0.13[b]
LV-2C-LS, %	124	-20.1 (-20.6, -19.7)	22	-22.2 (-23.3, -21.1)	0.001[b]
LV-GLS, %	118	-19.7 (-20.1, -19.3)	22	-21.3 (-22.2, -20.3)	0.004[b]
PSI-global LV, %	104	2.1 (1.5, 2.4)	21	1.8 (1.0, 2.5)	0.35[c]
PSI-LV-lateral wall, %	122	3.2 (2.62, 4.06)	22	3.3 (1.5, 5.2)	0.89[a]
PSI-LV-septal wall, %	126	2.0 (1.5, 2.4)	22	1.9 (0.9, 3.0)	0.98[a]
PSI-LV-anterior wall, %	117	1.2 (0.8, 1.6)	21	0.5 (-0.5, 1.4)	0.18[a]
PSI-LV-inferior wall, %	124	1.2 (0.9, 1.6)	21	1.2 (0.3, 2.0)	0.84[a]
PSI-LV-inferolateral wall, %	114	3.5 (2.6, 4.3)	21	3.1 (0.9, 5.2)	0.73[a]
PSI-LV-anteroseptal wall, %	117	1.6 (1.1, 2.1)	21	1.7 (0.4, 2.9)	0.94[a]
PSI-LV-all basal segments, %	114	2.9 (2.4, 3.4)	21	1.7 (0.4, 3.0)	0.11[a]
PSI-LV-all mid segments, %	118	1.2 (0.9, 1.4)	21	0.7 (0.1, 1.3)	0.20[a]
PSI-LV-all apical segments, %	108	2.4 (1.8, 2.9)	21	3.0 (1.6, 4.3)	0.46[a]
LV-segments with PSI > 0%, %	126	41%	22	33%	0.003[c]
RV-LS, %	121	-23.2 (-23.7, -22.6)	23	-23.3 (-24.6, -22.0)	0.84[b]
PSI-global RV, %	103	1.8 (1.2, 2.4)	18	2.3 (0.7, 3.8)	0.59[a]
RV-segments with PSI > 0%, %	120	30%	21	21%	0.04[c]
Peak VO_2, ml/kg/min	126	43.2 (41.4, 44.9)	22	48.6 (44.5, 52.6)	0.01[d]

a = ANCOVA adjusted for transducer dependent differences

b= ANCOVA adjusted for BSA and transducer dependent differences

c= chi-square test

d= ANCOVA adjusted for age, sex, and treadmill location dependent differences

CCS: childhood cancer survivor, CI: confidence interval, EF: ejection fraction, LV-4C-LS: left ventricular 4-chamber longitudinal strain, LV-3C-LS: left ventricular 3-chamber longitudinal strain, LV-2C-LS: left ventricular 2-chamber longitudinal strain, LV-GLS: left ventricular global longitudinal strain, PSI: post systolic index, RV-LS: right ventricular longitudinal strain, VO_2: oxygen consumption, ANCOVA: analysis of covariance, BSA: body surface area

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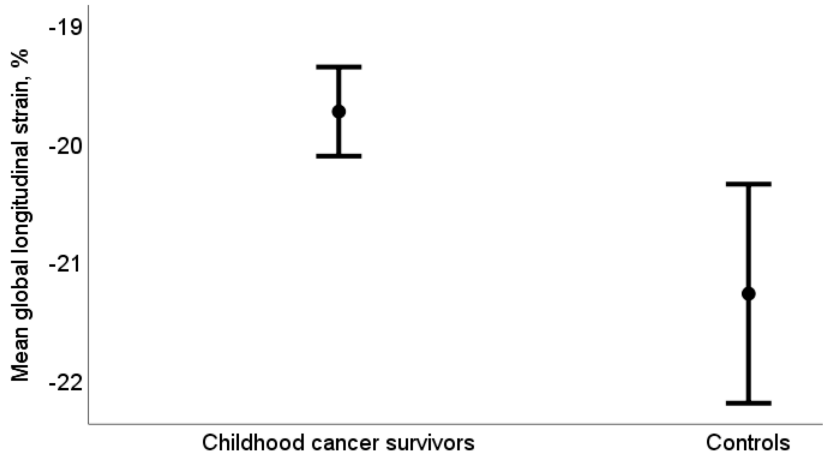
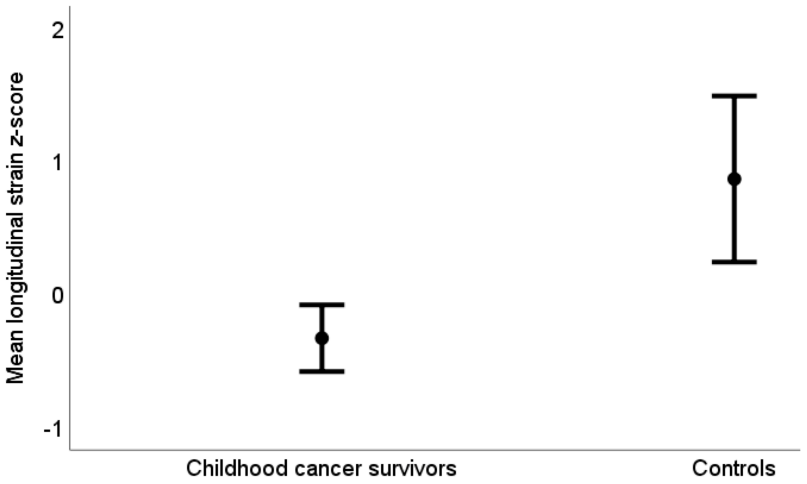
Figure 3
Left ventricle global longitudinal strain in childhood cancer survivors and controls



Mean left ventricular global longitudinal strain was -19.7% (95% confidence interval (CI) -20.1, -19.3) for childhood cancer survivors (n = 118) and -21.3% (95 % CI -22.2, -20.3) for controls (n = 22) (p = 0.004).

Figure 4
Left ventricle longitudinal strain z-score in childhood cancer survivors and controls



Mean left ventricular longitudinal z-score was -0.3 (95% confidence interval (CI) -0.6, -0.1) in childhood cancer survivors (n = 118) and 0.9 (95% CI 0.2, 1.5) in controls (n = 22) (p = 0.001).

Post systolic shortening

The presence of any degree of PSS (defined as PSI > 0%) was found more frequently in the wall-segments of CCSs compared to the wall-segments of controls (table 3).

Any degree of PSS was found in 41% (904 of 2192) of all the LV wall-segments in CCSs and in 33 % (127 of 384) of all the LV wall-segments in the controls ($p = 0.003$), and in 30% (213 of 700) of all the RV wall-segments in the CCSs and 21% (26 of 123) of all RV wall-segments in the controls ($p = 0.04$).

Mean global LV and RV PSI, and PSI in LV walls and regional wall-segments were similar in CCSs and controls (table 3).
Mean global LV PSI was 2.1% (95% CI 1.5, 2.4) for CCSs and 1.8% (95% CI 1.0, 2.5) for controls ($p = 0.4$), and mean global RV PSI was 1.8% (95% CI 1.2, 2.4) for CCSs and 2.3% (95% CI 0.7, 3.8) for controls ($p = 0.6$).

Chemotherapeutic agents and systolic myocardial function and NT-proBNP

Exposure to increasing doses of anthracyclines was associated with lower systolic myocardial function. PCC for EF Simpson was -0.2 ($p = 0.03$), for LV-GLS was 0.5 ($p < 0.01$), and for RV-LS 0.2 ($p = 0.04$). Correspondingly, NT-proBNP levels were positively associated with anthracycline dose with PCC 0.4 ($p < 0.001$). There was no significant association between increasing doses of Vincristine, Cisplatin or Carboplatin and measures of systolic LV or RV function.

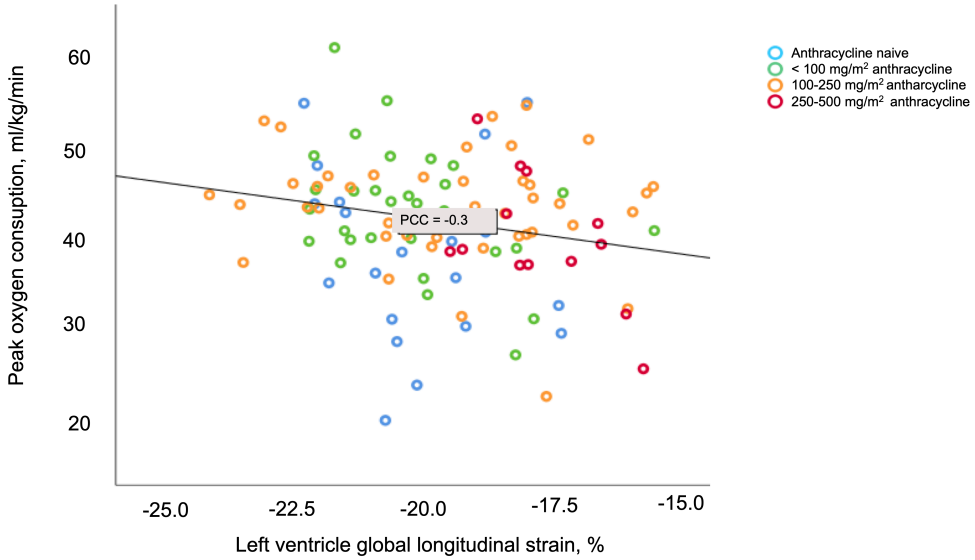
Radiation therapy and systolic myocardial function

Radiation therapy to the chest was associated with lower LV systolic function measured by EF Simpson and LV-GLS. However, in analysis adjusted for anthracycline doses, there was no significant association between radiation therapy and LV systolic function.

Peak oxygen consumption in the CCSs

Mean peak VO_2 was 43.2 ml/kg/min (95% CI 41.4, 44.9) in CCSs and 48.6 ml/kg/min (95% CI 44.5, 52.6) in the controls ($p = 0.01$).
Correlation analysis adjusted for sex, age, and transducer difference/treadmill location revealed a significant association between peak VO_2 and LV function measured by LV-GLS (PCC -0.3, $p = 0.01$) (figure 5) and EF Simpson (PCC 0.3, $p = 0.002$) in the CCSs, but no significant association between peak VO_2 and RV-LS or NT-proBNP level in the CCSs.

Figure 5
Peak oxygen consumption and left ventricular global longitudinal strain in childhood cancer survivors



Decreasing myocardial function measured by left ventricular global longitudinal strain was associated with decreasing peak oxygen consumption in childhood cancer survivors (Pearson correlation coefficient (PCC) -0.3, p = 0.01).

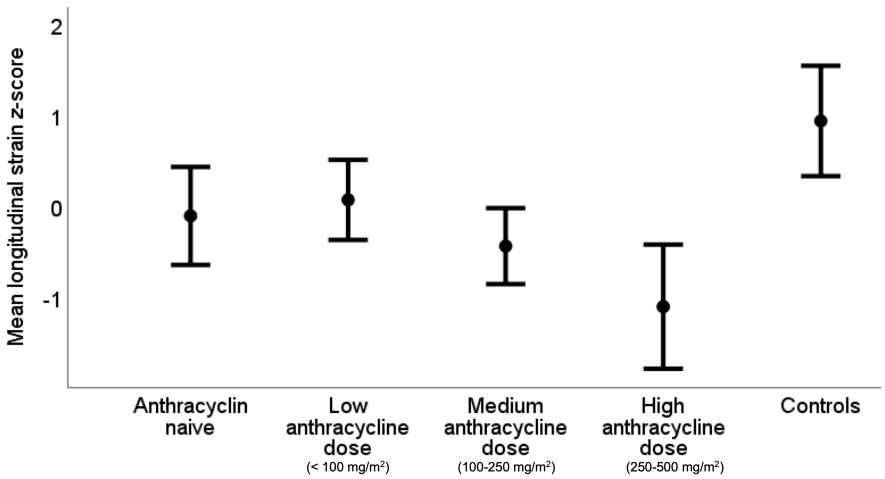
Subgroup analyses

We compared 4 subgroups of CCSs which were either anthracycline naive, had received low dose (< 100 mg/m^2), medium dose (100-250 mg/m^2), or high dose (250-500 mg/m^2) of anthracyclines (supplementary table 1). All four CCS subgroups included participants who had been treated with vinca alkaloids and/or platinum derivates. The subgroups were similar regarding age at examination time, sex and ethnicity distribution, BSA, BMI, resting blood pressure, time since diagnosis, and time after treatment.

Mean LV-GLS, and corresponding LV-LS z-scores, were significantly reduced in the subgroup treated with high dose of anthracyclines (-18.0% (95% CI -19.0, -17.0)) compared to the anthracycline naive subgroup (-20.3% (95% CI -21.1, -19.4)), p = 0.003, and the subgroups treated with low dose (-20.3% (95% CI -20.9,-19.6)), p = 0.001, and medium dose of anthracyclines (-19.7% (95% CI -20.4, -19.1)), p = 0.01, as described in supplementary table 1. Correspondingly, mean NT-proBNP level was significantly higher in the subgroup treated with high dose of anthracyclines (97 ng/L (SD 55)) compared to the anthracycline naive subgroup (54 ng/L (SD 30), p = 0.05, and the subgroup treated with low dose of anthracyclines (52 ng/L (SD 24)), p < 0.03. NT-proBNP was also higher in the subgroup treated with medium dose of anthracyclines (71 ng/L (SD 36)) compared to the subgroup treated with low dose of anthracyclines (p = 0.03). RV-LS, global LV PSI, and global RV PSI were similar in all four CCSs subgroups.

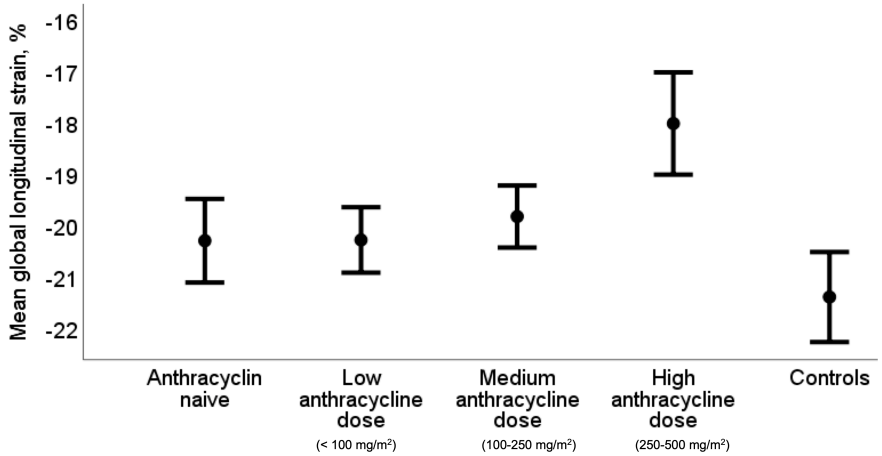
Z-score for LV-LS was lower in all four CCSs subgroups compared to the control group (all p-values ≤ 0.02) (figure 6), and mean LV-GLS was reduced in the two CCSs subgroups which received medium and high dose of anthracyclines compared to the control group (both p-values ≤ 0.03) (figure 7). RV-LS, global LV PSI, and global RV PSI were similar in all four CCSs subgroups compared to the control group.

Figure 6
Left ventricle longitudinal strain z-score in subgroups of pediatric childhood cancer survivors and controls



Mean left ventricular longitudinal strain z-score (LV-LS z-score) was impaired in all of the four childhood cancer survivor (CCS) subgroups compared to the control group (all p-values ≤ 0.02). For the anthracycline naive subgroup and the subgroups treated with low, medium, and high dose of anthracyclines z-score was -0.1 (95% confidence interval (CI) -0.6, 0.4), 0.1 (95% CI -0.4, 0.5), -0.4 (95% CI -0.8, -0.02), and -1.1 (95% CI -1.8, -0.4), respectively, versus 0.9 (95% CI 0.2, 1.5) in the controls.

Figure 7
Left ventricle global longitudinal strain in subgroups of pediatric childhood cancer survivors and controls



Left ventricular global longitudinal strain (LV-GLS) was significantly impaired in the childhood cancer survivor subgroups treated with high and medium dose of anthracyclines compared to the control group (p-values < 0.001 and p = 0.03, respectively)
LV-GLS was significantly impaired in the childhood cancer survivor subgroup treated with high dose of anthracyclines (-18.0% (95% confidence interval (CI) -19.0, -17.2)) compared to the anthracycline naive subgroup (-20.3% (95% CI -21.1, -19.4)) and the subgroups treated with low dose (-20.3% (95% CI -20.9,-19.6)) and medium dose of anthracyclines (-19.7% (95% CI-20.4, -19.1)), p = 0.003, p = 0.01, and p = 0.001, respectively.

Discussion

In this multicenter study, we found lower LV, but not RV, systolic function in 128 pediatric CCSs, compared to 23 healthy controls. Treatment with higher doses of anthracyclines and increasing time after treatment were associated with lower LV and RV systolic function, and lower LV myocardial function was associated with lower peak oxygen consumption during the cardiopulmonary exercise test.

Our finding of reduced LV-GLS in pediatric CCSs compared to controls indicates a detectable increased risk of developing heart failure in CCSs even at young age, and corresponds well to former findings in studies by Akam-Venkata et al. (11), Slieker et al. (13), and Yu et al. (33). The LV-LS z -score was < -2 in 13% of the CCSs, similar to the study by Slieker et al., which reported impaired LV longitudinal strain in 8% of 510 pediatric CCSs treated with similar mean anthracycline dose (150 mg/m^2) as our study participants (13).

Our subgroup- and correlation analyses revealed an inverse association between myocardial function measured by strain and anthracycline dose. This is in agreement with former knowledge of dose-dependent cardiovascular toxicity of cancer treatment (6). Worth noting is, however, that LV

function measured by LV-LS z-score was found reduced also in the anthracycline naive CCS participants, and in the subgroup treated with low doses of anthracyclines, compared to controls. This supports former reports of associations between vinca alkaloids or platinum derivates with heart failure (9, 34), and that there is probably no safe dose of anthracyclines (35). This might indicate that long term follow-up including echocardiographic evaluation of myocardial function should be considered in all CCSs, and not only in the subjects treated with moderate to high doses of potentially cardiotoxic chemotherapeutic agents (36).

While previous studies have reported global myocardial impairment after cancer disease and cardiotoxic treatment (11, 12, 37, 38), we found similar RV-LS in CCSs and controls. However, there was an association between increasing time after treatment and more reduced both LV-GLS and RV-LS, and we could suspect that the cardiotoxic effect might progress and cause RV dysfunction later in life. This is consistent with previous reports of progressive myocardial toxicity and reports of reduced RV function in adult CCSs, but not in pediatric CCSs (12, 13, 39).

Younger age at time of cancer treatment and female sex have been associated with reduced myocardial function in CCSs, but we found no such associations. This may be due to a relative moderate mean dose of anthracyclines (152 mg/m^2) in our study. Kramer et al (40) summarized ten studies from the late 90s to the early 2000s were the CCSs had been treated with mean or median anthracycline doses mainly above 250 mg/m^2. Of these, two studies reported an association between younger age at time of treatment and decreased myocardial function while four studies found the same association with female sex.

To further evaluate the cardiovascular risk and myocardial function in CCSs we investigated PSS in the myocardial wall-segments. Even though mean global PSI for both LV and RV were similar in CCSs and controls, we found a higher share of wall-segments with any degree of PSS in the CCSs compared to the controls. The mechanisms behind the finding of increased presence of PPS in the CCSs are uncertain but might be due to myocardial micro infarction related to both the cancer disease and/or its treatment. Even though any degree of PSS has been found in approximately 30% of LV wall-segments in healthy young adults (14), PSS has previously been reported to be a predictor of cardiovascular morbidity and death (17, 18). Our finding indicates that PSS might add as a marker of increased cardiovascular risk in the CCSs.

Similar to Wolf et al. (41), we found a positive association between anthracycline dose and NT-proBNP levels. However, even though the anthracycline dose was positively associated to NT-proBNP level and inversely associated to myocardial function, we found no significant association between NT-proBNP level and myocardial function. This is in agreement with former studies of NT-proBNP levels and myocardial function in CCSs (13, 42). However, as in our study, these studies included participants with mainly normal values of NT-proBNP. With the present cut-off values, diagnostic value of this biomarker to detect myocardial dysfunction in CCSs have been reported to be limited (43).

Only a few previous studies have investigated the relationship between myocardial function and the cardiorespiratory fitness in pediatric CCSs. Hogarty et al. (5) reported lower peak VO_2 in 33 pediatric CCSs, while Caro et al. (44) found similar peak VO_2 in 84 pediatric CCSs compared to controls. The participants in both studies had mainly normal myocardial function measured by echocardiographic fractional shortening. Other studies have reported reduced peak VO_2 in pediatric CCSs with reduced cardiac function revealed by myocardial imaging during exercise (4, 45), but also in CCSs with preserved myocardial stress response (4). Our study population is part of a larger study population

included in the PACCS study where 157 pediatric CCSs have participated in cardiopulmonary exercise testing (46). Bratteteig et al. reported a positive association between physical activity levels and peak VO_2 in these participants (46). In our sub-study, we found that lower LV myocardial function measured by EF Simpson and LV-GLS was associated with lower peak VO_2, likely due to reduced cardiac output (20), suggesting clinically significant reduction of LV myocardial function and reduced cardiac reserve in the CCSs. Although more complex mechanisms may also have contributed to the result, this corresponds well with former findings in adult CCSs where exercise intolerance and decreased myocardial function, and consequently reduced maximal oxygen consumption, are described as prevalent (2). These characteristics are associated with increased mortality (2) and are therefore important to identify in young CCSs. Additionally, as suggested by others, exercise testing might be useful to identify CCSs with subclinical myocardial impairment (41).

The strengths of this study were the study design with a well-matched control group, reducing the risk of confounding. The echocardiographic imaging was performed by highly trained sonographers, with the same ultrasound system, and all offline analyses were made by one cardiologist with the same echocardiographic software. The calculated intra- and inter-rater variability were good to excellent (47). There were limitations to the study. There is a risk of selection bias since extensive and physical challenging testing was part of the main study. This may have led to a selection of the fittest CCSs, which could most likely introduce an overestimate of LV or RV function in the CCSs group. EF and strain measurements are known to be afterload dependent with more reduced EF and longitudinal strain with higher blood pressure (48). We found significantly higher systolic blood pressure in the controls compared to the CCSs, which may have led to an underestimate of the difference in systolic myocardial function between CCSs and controls in this study. Peak VO_2 is mainly limited by the ability of the cardiorespiratory system to deliver oxygen to the exercising muscles (19), but other factors, such as respiratory or musculoskeletal dysfunction, deconditioning, or lack of motivation may also influence the result. Thus, complex mechanisms could have contributed to the association between decreasing myocardial function and decreasing peak oxygen consumption in the CCSs.

Regression analyses revealed significant influence of location in the echocardiographic measures and peak VO_2 results in the CCSs examined in Oslo versus those examined in Bergen, which could not be explained by difference in chemotherapy doses, radiation therapy, blood pressure or other characteristics. There might be technically or procedure related differences in exercise test results, as well as differences related to the ultrasound transducers used for echocardiographic imaging at the two centers. To address this issue as well as the single center inclusion of controls, correction for location was added in the statistical analyses. The limited number of controls in the study is also considered a limitation.

To conclude**,** we found reduced left ventricular systolic myocardial function in pediatric CCSs compared to healthy controls, even in participants treated with low doses of anthracyclines. Lower left ventricular function was associated with lower peak VO_2, suggesting reduction of left ventricular function of clinically significance. The right ventricular function was similar in CCSs and controls suggesting regional differences regarding the impact of the cardiotoxic treatment on myocardial function. Increasing dose of anthracyclines and increasing time after treatment were, however, associated with lower myocardial function of both the left and right ventricle. This implies that long-

term follow-up, including evaluation of myocardial function, and tight control of modifiable cardiovascular risk factors might be indicated for all childhood cancer survivors.

Abbreviations

CCCs: childhood cancer survivors
EF: ejection fraction
LV-GLS: left ventricular global longitudinal strain
LV-LS: left ventricular longitudinal strain (derived from 4-chamber view)
NT-proBNP: N-terminal pro-Brain Natriuretic peptide
PACCS: Physical Activity and fitness in Childhood Cancer Survivors
PCC: Person correlation coefficient
PSI: post systolic index
PSS: post systolic shortening
RV-LS: right ventricular longitudinal strain (derived from lateral wall and septum)
VO_2: oxygen consumption

Contribution to the field statement

Cardiovascular disease is a leading non-cancer contributor to early morbidity and mortality in childhood cancer survivors (CCSs). Myocardial function measured by echocardiographic strain and the physiologic response of the cardiorespiratory system to exercise have mainly been investigated in adult survivors of childhood cancer. However, evaluation of cardiac function and fitness in pediatric CCSs is of importance to identify cardiovascular risk at an early point. We report findings of reduced myocardial function in pediatric CCSs compared to age- and sex matched controls, and an association between lower myocardial function and lower peak oxygen consumption in CCSs suggesting impairment of myocardial function of clinical significance. Increasing anthracycline dose was associated with lower myocardial function, however, myocardial function was found impaired also in participants treated with low doses of anthracycline and in anthracycline naive CCS participants. Additionally, increasing time after treatment was associated with lower myocardial function. Our findings might indicate that long-term follow up, including evaluation of myocardial function, is important in all CCSs, including subjects exposed to low doses of potentially cardiotoxic treatment.

Conflict of interest statement

The authors have no relevant financial or non-financial interests to disclose.

Authors contribution statement:

BE, SD, HB, TR, IT, TRO, GG, ER, EE, and EL contributed to the study conception and design. Material preparation, data collection and analysis were performed by BE, SD, HB, TR, IT, TRO, ER, and EE. FZG gave advice regarding statistical analyses. All authors participated in the interpretation of the data, and critically reviewed and commented on the first and previous drafts of the manuscript written by BE. All authors approved of the final manuscript.

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Data availability statement

In accordance with the approvals granted for this study by The Regional Committee on Medical Research Ethics and The Norwegian Data Protection Authority, the data files are stored securely and in accordance with the Norwegian Law of Privacy Protection. The data file cannot be made publicly available without a risk of identifying anonymous participants and thereby compromise the respondents' privacy. To prepare future research papers other researchers in our group currently use the data file. A subset of the data file with anonymized data from this sub-study of the PACCS study can be made available to interested researchers upon reasonable request to Britt Engan (Britt. Engan@Helse-Bergen.no), providing Norwegian privacy legislation and GDPR are respected, and that permission is granted from The Norwegian Data Protection Authority and the data protection officer at Oslo University Hospital.

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Supplementary table 1
Characteristics and measures of myocardial systolic function and peak oxygen consumption in childhood cancer survivors by subgroups

	CCSs Anthracycline naive n=26	CCSs Anthracycline dose <100mg/m² n=36	CCSs Anthracycline dose 100-250mg/m² n=44	CCSs Anthracycline dose 250-500mg/m² n=15	p-value	Controls n=23
Sex, males, n (%)	16 (62%)	16 (44%)	24 (55%)	6 (40%)	0.53[a]	10 (43%)
Ethnicity, Caucasian, n (%)	24 (92%)	32 (89%)	41 (93%)	14 (93%)	0.97[a]	23 (100%)
Ethnicity, Asian, n (%)	1 (4%)	1 (3%)	1 (2%)	1 (7%)		
Ethnicity, mixed, n (%)	1(4%)	2 (5%)	2 (5%)	0		
Ethnicity, other, (%)	0	1 (3%)	0	0		
Age at study, years, mean (SD)	13.6 (2.4)	12.8 (2.7)	14.0 (2.6)	14.0 (2.7)	0.20[b]	12.7 (3.1)
Age at diagnosis, years, mean (SD)	6.1 (3.9)	4.2 (2.6)	5.4 (3.5)	6.2 (3.4)	0.07[c]	
Time since diagnosis, years, mean (SD)	7.4 (3.7)	8.7 (3.1)	8.6 (3.5)	8.0 (4.1)	0.50[b]	
Time since last treatment, years, mean (SD)	5.9 (3.6)	6.3 (2.9)	7.0 (3.5)	7.1 (4.1)	0.53[b]	
BMI, kg/m², mean (95% CI)	20.3 (18.8, 21.9)	19.5 (18.2, 20.8)	20.5 (19.3, 21.6)	20.5 (18.5, 22.5)	0.68[d]	19.0 (17.5,20.5)
BSA, m², mean (95% CI)	1.5 (1.4, 1.6)	1.4 (1.3, 1.5)	1.5 (1.5, 1.6)	1.5 (1.4, 1.6)	0.34[d]	1.4 (1.3, 1.5)
Systolic BP, mmHg, mean (95% CI)	108 (104,112)	106 (102,109)	108 (105, 111)	107 (102, 112)	0.84[d]	112 (108, 116)
Diastolic BP, mmHg, mean (95% CI)	65 (62,68)	64 (61,67)	67 (65, 70)	66 (62,71)	0.56[d]	69 (65,73)
NT-proBNP, ng/L, mean (SD)	54 (30)	52 (24)	71 (36)	97 (55)	0.004[c]	
Hb, g/dL, mean (SD)	13.3 (1.2)	13.5 (0.9)	13.6 (1.0)	13.4 (0.9)	0.8[b]	
Anthracyclines, n	0	36 (100%)	44 (100%)	15 (100%)		
Anthracycline dose, mg/m², mean (SD)	0	79 (6)	152 (38)	329 (63)		
Vinca alkaloids, n (%)	24 (92%)	35 (97%)	41 (93%)	13 (87%)		
Vincristine, n	21	35	41	13		
Vincristine dose, mg/m², mean (SD)	32 (22)	30 (5)	26 (12)	21 (10)		
Vinblastine, n	3	0	0	0		
Vinorelbine, n	0	0	1	0		
Platinum derivates, n (%)	15 (58%)	1 (3%)	6 (14%)	5 (33%)		
Cisplatin, n	8	1	6	1		
Cisplatin dose, mg/m², mean (SD)	247 (155)		327 (95)			
Carboplatin, n	13	1	6	4		
Carboplatin dose, mg/m², mean (SD)	4734 (3581)		1919 (1372)	3199 (282)		
Radiation chest, n	0	0 (0%)	3 (7%)	2 (13%)		
EF Simpson, %, mean (95% CI)	58 (57, 60)	62 (60, 63)	60 (59, 62)	57 (55, 60)	0.01[d]	63 (61, 66)
LV-GLS, %, mean (95% CI)	-20.3 (-21.1, -19.4)	-20.3 (-20.9, -19.6)	-19.7 (-20.4, -19.1)	-18.0 (-19.0, -17.0)	0.002[d]	-21.3 (-22.2, -20.3)
LV-4C-LS, %, mean (95% CI)	-19.7 (-20.5, -18.9)	-19.9 (-20.6,-19.2)	-19.2 (-19.9, -18.6)	-18.2 (-19.2, -17.2)	0.04[d]	-21.2 (-22.1, -20.2)
Z-score LV-LS, mean (95% CI)	-0.1 (-0.6, 0.4)	0.1 (-0.4, 0.5)	-0.4 (-0.8, -0.02)	-1.1 (-1.8, -0.4)	0.03[d]	0.9 (0.2, 1.5)
RV- LS, %, mean (95% CI)	-23.6 (-24.7, -22.4)	-23.5 (-24.4, -22.6)	-22.9 (-23.8, -22.0)	-21.9 (-23.4, -20.4)	0.27[d]	-23.3 (-24.6, -22.0)
PSI-global LV, %, mean (95% CI)	2.1 (1.4, 2.8)	2.0 (1.5, 2.5)	2.2 (1.7, 2.7)	2.6 (1.8, 3.5)	0.68[d]	1.8 (1.0, 2.5)
PSI-global RV, %, mean (95% CI)	1.5 (-0.2, 3.2)	1.6 (0.5, 2.8)	2.1 (1.0, 3.2)	2.4 (0.5, 4.3)	0.84[d]	2.3 (0.7, 3.8)
Peak VO_2, ml/kg/min mean (95% CI)	38.0 (34.5, 41.4)	44.7 (41.7, 47.8)	44.7 (41.9, 47.5)	39.6 (35.0, 44.2)	<0.01[d]	48.6 (44.5, 52.6)

a= Chi-square test or Fisher`s exact test

b= classic ANOVA, post hoc Tukey

c= Welch´s ANOVA, post hoc Games-Howell

d= ANCOVA (with Sidak correction) adjusted for age, sex, BSA, echocardiographic transducer difference and treadmill location as appropriate.

CCS: childhood cancer survivor, SD: standard deviation, CI: confidence interval, BMI: body mass index, BSA: body surface area, BP: blood pressure, NT-proBNP: N-terminal pro-Brain Natriuretic peptide, Hb: hemoglobin, CI: confidence interval, EF: ejection fraction, LV-GLS: left ventricular global longitudinal strain, LV-4C-LS: left ventricular 4-chamber longitudinal strain, PSI: post systolic index, RV-LS: right ventricular longitudinal strain, VO_2: oxygen consumption, ANOVA: analysis of variance, ANCOVA: analysis of covariance

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Errata for

Cardiac Function and Early Risk Markers for Cardiovascular Disease in Children and Young Adults Surviving Severe Childhood Disease

Britt Engan

Thesis for the degree philosophiae doctor (PhD)
at the University of Bergen

01.10.23 Britt Engan
(date and sign. of candidate)

02.10.23
(date and sign. of faculty)

Errata

Page 65 Wrong unit of measurement for body surface area (BSA): "kg/m^2" – corrected to "m^2"

Page 39 and 62 Misspelling author name: "Russel" – corrected to "Russell"

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