Altered body composition in adults with complex congenital heart disease

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To Elias
Wherever you go, go with all your heart

Confucius
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Original Papers

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


III **Johansson K**, Johansson B, Sandberg C. Grip strength is a good marker of sarcopenia in adults with complex congenital heart disease (Submitted)

IV **Johansson K**, Johansson B, Sandberg C. Reduced bone strength in adults with complex congenital heart disease (Submitted)

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Abstract

Background

Thanks to achievements in paediatric heart surgery and medicine, the population of adults with surgically repaired or palliated congenital heart defects is growing. Many of these adults have reduced exercise capacity, weaker muscular strength and shorter height, all of which suggest an altered body composition.

The overall aim of this thesis was to evaluate the body composition, in terms of bone, muscle and fat mass, in adults with complex congenital heart disease (CHD). Changes as such may be of prognostic importance and thus suggest future therapeutic targets outside the traditional hunting grounds of the cardiologist.

Material and methods

The overall material consisted of two cohorts. The first cohort, recruited in a Swedish multicentre study, comprised 73 adult patients with complex CHD and 73 controls, matched for age and sex. Participants were examined with full body dual-energy x-ray absorptiometry (DXA), providing muscle, bone and fat mass for arms, legs and trunk respectively (papers I and II).

The second cohort, recruited within a single centre study, comprised 49 adult patients with complex CHD and 49 age and sex matched controls. Participants were examined with peripheral quantitative computed tomography (pQCT), providing slices of forearm and calf, describing muscle, bone and fat area and corresponding density (papers III and IV).

Muscular strength in selected muscle groups was also evaluated in both cohorts.

Results

More than half of the adults with complex CHD had a pathologically low skeletal muscle mass and strength compared to controls, a trait referred to as
There was a strong association between forearm muscle mass and grip strength.

Bone mass was lower in adults with complex CHD, according to both DXA and pQCT analyses, also when adjusting for shorter height. Patients also had lower full body bone mineral density (BMD) as measured with DXA. However, analysis of BMD in limbs with pQCT showed no such reduction. Despite this latter finding, the strength-strain index (a surrogate marker for bone strength provided by pQCT in the lower limbs) was still lower in patients compared to controls.

Female patients had a higher amount of fat, both in terms of fat mass and proportion of fat, in comparison to controls. The fat mass was predominantly distributed around the internal organs, known as visceral adipose tissue. Male patients showed no such difference regarding fat mass compared to controls.

**Conclusion**

Consequences of living with complex CHD go far beyond the heart; this young population presents a reduced skeletal muscle mass as well as reduced bone strength – both premature traits of frailty, prone to increase with further ageing. Also, women with complex CHD have an increased amount of visceral adipose tissue, which may elevate the risk of acquired heart disease.

The extent of future complications remains to be seen. However, the standard treatments for both sarcopenia and osteoporosis include optimal nutritional intake and increased physical exercise. These measures should start sooner rather than later, preferably evaluated through existing quality registers and interventional trials.
## Abbreviations

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<td>ACHD</td>
<td>Adult Congenital Heart Disease</td>
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<td>AFM</td>
<td>Appendicular Fat Mass</td>
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<tr>
<td>ALM</td>
<td>Appendicular Lean Mass</td>
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<td>ALM-index</td>
<td>Appendicular Lean Mass-index</td>
</tr>
<tr>
<td>AV</td>
<td>Atrio-Ventricular</td>
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<tr>
<td>BF</td>
<td>Body Fat</td>
</tr>
<tr>
<td>BF%</td>
<td>Body Fat percentage</td>
</tr>
<tr>
<td>BMI</td>
<td>Body Mass Index</td>
</tr>
<tr>
<td>BMC</td>
<td>Bone Mineral Content</td>
</tr>
<tr>
<td>BMD</td>
<td>Bone Mineral Density (pQCT)</td>
</tr>
<tr>
<td>BMDa</td>
<td>areal Bone Mineral Density (DXA)</td>
</tr>
<tr>
<td>cc-TGA</td>
<td>congenitally corrected Transposition of the Great Arteries</td>
</tr>
<tr>
<td>CHD</td>
<td>Congenital Heart Disease</td>
</tr>
<tr>
<td>CSA</td>
<td>Cross-Sectional Area</td>
</tr>
<tr>
<td>d-TGA</td>
<td>dextro Transposition of the Great Arteries</td>
</tr>
<tr>
<td>DXA</td>
<td>Dual-energy X-ray Absorptiometry</td>
</tr>
<tr>
<td>FM</td>
<td>Fat Mass</td>
</tr>
<tr>
<td>FM-index</td>
<td>Fat Mass-index</td>
</tr>
<tr>
<td>IPAQ</td>
<td>International Physical Activity Questionnaire</td>
</tr>
<tr>
<td>LM</td>
<td>Lean Mass</td>
</tr>
<tr>
<td>MET</td>
<td>Metabolic Equivalent of Task</td>
</tr>
<tr>
<td>pQCT</td>
<td>peripheral Quantitative Computed Tomography</td>
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<tr>
<td>RVOT</td>
<td>Right Ventricular Outflow Tract</td>
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<tr>
<td>SSI</td>
<td>Strength-Strain Index</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>TCPC</td>
<td>Total Cavo-Pulmonary Connection</td>
</tr>
<tr>
<td>VAT</td>
<td>Visceral Adipose Tissue</td>
</tr>
<tr>
<td>VSD</td>
<td>Ventricular Septal Defect</td>
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</table>
Definitions in short

Appendicular  Relating to the limbs, i.e. arms and legs
Artery  A vessel that conducts blood away from the heart
Atrium  The upper heart chambers through which the blood enters the ventricles
Baffle  Surgically created intra-atrial wall that makes up a channel to divert blood flow
Body composition  The study of components of the body; can be done on different levels. In this thesis on the level of muscle, bone and fat
Concentric  In terms of muscle contractions: a shortening
Conduit  A channel for passage of fluids; in CHD this refers to an inserted tube or tunnel to conduct blood in association with the heart. Could include a valve
Congenital  Present at birth, in contrast to acquired
Cortex  The dense outer surface of the bone
Cyanosis  Haemoglobin with a low oxygen saturation, causing a bluish discoloration. In CHD usually due to a shunt from right to left side of the heart
Diaphysis  The shaft of a long bone
Distal  Situated away from the centre of the body, as opposed to proximal, e.g. fingers are distal to the hand
Ductus arteriosus  Foetal connection between the aorta and the pulmonary artery
Eccentric  In terms of muscle contraction: a lengthening
Epiphysis  The rounded end part of a long bone
Graft  Surgically implanted or transplanted tissue, usually tubular. The first grafts were biological in origin. A xenograft is animal in origin and a homograft is human in origin
<table>
<thead>
<tr>
<th>Term</th>
<th>Definition</th>
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<tr>
<td>Isometric</td>
<td>In terms of muscle contractions: a static contraction of a muscle, i.e. with no noticeable change in length</td>
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<tr>
<td>Lean mass</td>
<td>Lean body mass; total body weight minus bone and fat mass; a proxy for skeletal muscle mass</td>
</tr>
<tr>
<td>Ligature; ligate</td>
<td>Thread or wire used to constrict tissue; to apply a ligature</td>
</tr>
<tr>
<td>Marrow</td>
<td>Soft tissue in the centre of the bone</td>
</tr>
<tr>
<td>Morbidity</td>
<td>Rate of disease</td>
</tr>
<tr>
<td>Mortality</td>
<td>Death rate</td>
</tr>
<tr>
<td>Paediatric</td>
<td>The medical care of infants, children, and adolescents</td>
</tr>
<tr>
<td>Proximal</td>
<td>Close to the centre of the body, as opposed to distal</td>
</tr>
<tr>
<td>Shunt</td>
<td>Non-physiological flow of blood. Could be through a congenital, acquired or surgically created connection between heart chambers or blood vessels</td>
</tr>
<tr>
<td>Systemic</td>
<td>In normal physiology this refers to the aorta (distributing blood to the whole body), as opposed to pulmonary. The “systemic ventricle”, (not necessarily a morphological left ventricle), pumps the oxygenated blood to the aorta in contrast to the “sub pulmonary” ventricle, pumping blood through the lungs</td>
</tr>
<tr>
<td>Systolic</td>
<td>The phase in the heartbeat cycle when the heart muscle contracts, as opposed to diastolic</td>
</tr>
<tr>
<td>Teratogen</td>
<td>An agent or factor that causes malformations of an embryo</td>
</tr>
<tr>
<td>Trabecular bone</td>
<td>Porous bone in the centre of the bone, mainly at the end of the tubular bones</td>
</tr>
<tr>
<td>Vein</td>
<td>A vessel that conducts blood towards the heart</td>
</tr>
<tr>
<td>Ventricle</td>
<td>The muscular chamber that pumps the blood out of the heart</td>
</tr>
<tr>
<td>Visceral</td>
<td>Related to the internal organs, e.g. visceral adipose tissue around the organs of the abdomen</td>
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Enkel sammanfattning på svenska

Bakgrund

Tack vare stora framsteg inom barnhjärtkirurgin överlever allt fler barn med medfödda hjärtfel, så kallade hjärtebarn, till vuxen ålder. Många av dessa vuxna med medfödda hjärtfel har nedsatt kondition, nedsatt styrka och lite kortare längd – vilket väcker misstanken om avvikande kroppssammansättning.

Syftet med avhandlingen var att kartlägga kroppssammansättningen hos vuxna med komplexa medfödda hjärtfel, då dylika förändringar kan påverka prognos och behandling.

Material och metod

Materialet bestod av två kohorter: den första kom från en svensk multicenterstudie med 73 vuxna med komplexa medfödda hjärtfel och 73 åders- och könsmatchade kontroller. Dessa genomgick helkroppssökning med dual-energy x-ray absorptiometry (DXA), en metod för att mäta muskel-, ben- och fettmassa. (artikel I och II)

Den andra kohorten kom från en singelcenterstudie, innefattande hela norra regionen, med 49 patienter och likaledes matchade kontroller. Dessa undersöktes med peripheral quantitative computed tomography (pQCT) som genom datortomografiska snitt av underarm och underben kartlägger muskel-, ben- och fettarea. (artikel III och IV)

Resultat

Hälften av patienterna med komplexa medfödda hjärtfel hade sänkt muskelmassa och muskelstyrka (sarkopeni), vilket ofta förknippas med försämrad prognos. Detta sågs både i studien med DXA och studien med pQCT.

Båda metoderna visade även sänkt benmassa. DXA visade på sänkt bendensitet, något som inte kunde bekräftas med pQCT. Däremot uppmättes sämre hållfasthet i skelettet med den senare metoden.
Kvinnliga patienter hade större andel fett än de manliga, framför allt distribuerat i buken, vilket är extra ohälsosamt.

**Slutsats**


Hur mycket den avvikande kroppssammansättningen kommer att påverka sjuklighet och överlevnad när vuxna med komplexa medfödda hjärtsel åldras får framtida studier utvisa. Men utifrån erfarenheter från andra patientgrupper finns det ingen anledning att vänta med förebyggande behandling mot såväl sarkopeni och benskörhet som förvärvad hjärtsjukdom – närmare bestämt uppmuntra till följsamhet till befintliga kosträdd samt motion.
Background

The early history of congenital heart disease

The heart has always held a great interest to mankind. Its anatomy was dissected by great minds like Leonardo da Vinci\(^1\), and pathological variations were described in systematic detail since the 19th century\(^2\); an example is seen in figure 1.

However, until the second half of the 20\(^{th}\) century, close to nothing could be done for children with congenital heart defects. Bluish children grasping for air, and the doctor recognising a murmur of the heart, was the worst fear of many parents. Diagnostic tools were clinical examination, electrocardiography and chest x-ray. Treatment was often limited to bedrest and morphine to ease the suffering\(^3\).

Before the 1950s, the patients that survived open-heart surgery were few, and interventions, with few exceptions, were restricted to the exterior of the heart\(^4\). For instance, Mr Dwight Harken developed techniques to close penetrating heart wounds during World War II\(^5\). In the case of congenital heart defects, Dr Robert Gross set a milestone when succeeding to ligate a patent ductus arteriosus in 1938\(^6\). This persisting foetal connection between the aorta and pulmonary artery, could eventually result in a fatal volume overload of the pulmonary circulation and the left side of the heart. The closure of the patent ductus arteriosus is considered by some as the beginning of the era of heart surgery and was followed by inventions of different shunts and vascular interventions. One such intervention was the first repair of a coarctation of the aorta by Swedish surgeon Clarence Crafoord in 1944\(^7\). But

![Figure 1 An open foramen ovale, illustrated in “On malformations etc., of the human heart” by Thomas Peacock in 1858.](image)

\(^1\)Da Vinci, Leonardo
\(^2\)Pathological variations described since the 19th century
\(^3\)Treatment limited to bedrest and morphine
\(^4\)Surviving open-heart surgery rare
\(^5\)Techniques for closing penetrating heart wounds during World War II
\(^6\)Ligation of patent ductus arteriosus in 1938
\(^7\)First repair of aortic coarctation by Clarence Crafoord in 1944
still, surgeons had only been scratching the surface of the heart. The greatest challenge remained: being able to operate inside the heart.

The inside of a pumping, blood-filled heart seemed impossible to reach without killing its carrier. Surgeons tried multiple approaches, and many were, literally, a dead end. The heart and lungs had to be bypassed using a machine doing the pumping and oxygenation. Some surgeries were performed using animal lungs that sometimes unfortunately clogged. Others were done using the so-called cross-circulation where a parent’s bloodstream was connected to and supporting the child’s circulation during heart surgery. The technique proved to work, but had the unthinkable outcome of killing both parent and child – a fatality ratio of 200 \%^{3,8}.

Perhaps it is safe to say the biggest leap in the history of paediatric heart surgery was not a single surgical technique but the invention of the heart-lung machine during the 1950s. Fully bypassing the heart and lungs with a pump and an oxygenating chamber allowed surgeons to operate with good visibility and on a non-beating heart for, in this context, longer time periods. The era of paediatric heart surgery had begun\textsuperscript{9}.

**The heart**

The normal physiology of the healthy heart is simple – two muscular pumps in a row, as illustrated in figure 2. The right pump, or sub pulmonary ventricle, receives venous blood from the body and pumps it through the lungs for oxygenation. The left ventricle receives the oxygenated blood from the lungs and pumps it forward through the aorta out into the body. Prior to the ventricles are the atria that are interconnected with atrio-ventricular (AV) valves that prevents blood from flowing in the wrong direction\textsuperscript{10}.

In the eyes of a paediatric cardiologist, the morphological right ventricle has the following traits: (1) trabeculated inner wall, (2) moderator band, (3) apical displacement of the AV-valve and (4) a muscle bundle between the AV-valve and the pulmonary valve, the supraventricular crest. In figures 2-10, the morphological right ventricle is symbolized by a trabeculated inner wall.
Congenital heart disease

Congenital heart disease (CHD) is a structural defect present at birth in the walls, valves or great vessels of the heart. CHD is the most common birth defect, affecting approximately 1% of all live born children. In Sweden, the estimated incidence is 750-1000/year\textsuperscript{11}. The aetiology is mainly unknown with only approximately 20% of cases being explained by chromosomal abnormalities such as Downs syndrome, other genetic disorders or teratogens\textsuperscript{12}.

Figure 2 Normal heart. Unoxygenated blood from the superior and inferior vena cava (SVC, IVC) enters the right atrium (RA), passing the tricuspid valve (TV) to the right ventricle (RV). From the right ventricle, blood passes the pulmonary valve (PV) to the pulmonary artery (PA) and further to the lungs. Oxygenated blood from the lungs enters the left atrium (LA) via the pulmonary veins, passes the mitral valve (MV) to the left ventricle (LV). From the left ventricle, blood is pumped through the aortic valve (AV) and the aorta (Ao) to the rest of the body. Note the thicker wall of the left ventricle.

The complex lesions

Congenital heart lesions can be classified according to morphology, presence of cyanosis or severity of lesions. Throughout the thesis, I have focused on the complex lesions as classified by Eriksen et. al \textsuperscript{13}.

The complex lesions comprise combinations of several defects and include all cyanotic lesions. The following is a brief description and illustration of the complex CHD included in this thesis and the main surgical interventions, which by no means claims to be complete\textsuperscript{10, 14}.
Tetralogy of Fallot

Tetralogy of Fallot is named after Etienne Louis Arthur Fallot, a French forensic pathologist who anatomically described the defect in 1888. Tetralogy of Fallot is the combination of ventricular septal defect (VSD), obstruction of the right ventricular outflow tract (RVOT), hypertrophy of the right ventricle and an overriding aorta (figure 3). Overriding aorta is when the aorta and aortic valve is pulled to the right and situated over the interventricular septum. If the right ventricular outflow tract and pulmonary valve is not only narrowed but completely obstructed, the condition is equal to pulmonary atresia with VSD.

To allow for an increased amount of blood to flow through the lungs, despite the obstructed pulmonary valve, the subclavian artery was connected to the pulmonary artery, a procedure first performed in 1944 known as the Blalock-Taussig shunt. This was the only procedure known to ease the symptoms for patients with Tetralogy of Fallot in the era before the heart lung machine, and it is still sometimes used to bridge infants to corrective surgery and allows for development of too small pulmonary arteries. Nowadays, the procedure is
modified using a Gore-Tex graft. The first correction of a Tetralogy of Fallot was performed in 1954 and comprised repair of the VSD with a patch, resection of muscle bundles in the RVOT and a patch to widen the RVOT; this was performed by the legendary surgeon Walter Lillehei\textsuperscript{17}.

According to some classifications, Tetralogy of Fallot is considered as a moderately complex CHD\textsuperscript{18}. However, since patients with Tetralogy of Fallot have a low physical performance, in line with other complex lesions described below, they are include among the complex lesions in this thesis.

**Pulmonary atresia with intact ventricular septum**

Pulmonary atresia is quite rare and means an undeveloped pulmonary valve or pulmonary artery (figure 4). If the ventricular septum is intact, unoxygenated blood passes from the right atrium to the left side of the circulation via a persistent foramen ovale between the two atria. The defect is depending on the ductus arteriosus and foramen ovale to stay open, and requires palliative procedures to be performed within days after birth\textsuperscript{10}.

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**Figure 4** Pulmonary atresia with intact ventricular septum. Note the undeveloped pulmonary valve (PV) and unoxygenated blood passing between the atrias through a persistent foramen ovale (PFO) as well as the passing of blood from the aorta to the pulmonary artery through a patent ductus arteriosus (PDA).
Further surgery depends on the severity of the defect and could be similar to the Tetralogy of Fallot procedure described above. If the right ventricle is too small and underdeveloped, it might be preferred to proceed with a univentricular strategy as described below.

Additionally, pulmonary atresia is often complicated by coronary fistulae draining into the right ventricle. If the right ventricular pressure falls, a coronary steal phenomenon may occur that results in myocardial ischemia.

**Truncus arteriosus**

If the pulmonary artery and the aorta consist of a common trunk with a common valve, the defect is called truncus arteriosus. In truncus arteriosus, there is usually a VSD and often other associated malformations of valves and the great vessels (figure 5). The lesion is often subdivided into four types, based on the pulmonary artery anatomy\(^1\).

The first ever successful repair of truncus arteriosus was performed in 1963\(^2\). Nowadays, corrective surgery allows the truncal valve to serve as the aortic valve where a conduit with a valve is placed between the right ventricle and the pulmonary artery.
**Transposition of the great arteries**

When the pulmonary artery arises from the left ventricle and the aorta arises from the right ventricle, the condition is called dextro transposition of the great arteries (d-TGA)(figure 6). In d-TGA, unoxygenated blood from vena cava and oxygenated blood from the lungs would be circulating completely separated from each other in the absence of a persistent foramen ovale or patent ductus arteriosus\(^21\).

The original correction procedure involved several steps. First, a shunt between the right and left atria had to be created, such as the Blalock-Hanlon atrial septectomy that was first performed in 1950\(^22\). This open-heart surgery was later replaced by an endovascular balloon septostomy known as the Rashkind procedure that was first performed in 1966\(^23\). Second, blood from the left atrium was redirected to the right ventricle via intraatrial channels separated by a baffle – a so-called atrial switch. The first atrial switch used the atrial wall as a baffle, the so-called Senning procedure, and was first performed in Stockholm by Åke Senning in 1957\(^24\).

The operation was technically demanding and a few years later, a synthetic material was used to create the baffle, known as a Mustard procedure; this was...
first performed in Toronto by William Mustard in 1963\textsuperscript{25}. The problem with both operations was that the anatomical right ventricle was left in the systemic position, thereby limiting aerobic capacity and leading to the eventual failure of the ventricle. Furthermore, the extensive atrial surgery makes the patients prone to atrial arrhythmias. Therefore, surgeons struggled with switching the aortic and pulmonary artery instead – a so-called arterial switch. The first arterial switch was performed in 1975, but due to considerable problems with perioperative complications such as myocardial infarction due to difficulties to handle re-implantation of the coronary arteries, it was not until the 1990s the procedure became a standard of care\textsuperscript{25}.

**Congenitally corrected transposition of the great arteries**

If the left ventricle is pumping blood to the lungs via the pulmonary artery, and the returning oxygenated blood is pumped by the right ventricle to the aorta, the condition is referred to as congenitally corrected transposition of the great arteries (cc-TGA)(figure 7). However, the term ‘congenitally corrected’ is somewhat misleading since no corrective surgery has been performed. In fact, cc-TGA could remain undetected due to a physiological circulation and absence of cyanosis, and is therefore often undiagnosed for many years\textsuperscript{21}.

![Figure 7 Congenitally corrected transposition of the great arteries.](image)
Patients with cc-TGA may sometimes be operated with a so-called double switch, a Senning/Mustard procedure followed by an arterial switch. The double switch is a more physiological solution when the left ventricle works as the systemic high-pressure ventricle, instead of the right ventricle, that is intended for the low pressure in the pulmonary circulation. However, many patients remain undiagnosed during childhood and miss out on palliative surgery. Also, the double switch is a complicated procedure that is used only in selected patients.

**Univentricular heart and the Fontan circulation**

The group univentricular heart is heterogenous and comprise multiple defects, all sharing the presence of only one functional ventricle. Defects with univentricular heart could be, for instance, mitral or tricuspid atresia, hypoplastic left heart syndrome or double inlet ventricles.

When biventricular repair is not possible, a sequence of interventions is preferred to connect the central venous return directly to the pulmonary arteries and the single ventricle to act as a systemic ventricle below the aorta. Thereby, the pulmonary circulation is driven only by the transpulmonary pressure gradient. The advantage is that volume overload is relieved from the ventricle. Furthermore, the haemoglobin saturation usually returns to the

![Figure 8 The original Fontan procedure as described in the original article from 1971. Reproduced from Surgical repair of tricuspid atresia, F. Fontan and E. Baudet, Thorax volume 26, page 240, copyright BMJ 1971, with permission from BMJ Publishing Group Ltd.](image-url)
normal range. The single ventricle physiology is referred to as Fontan circulation, named after Dr. Francois Fontan, the surgeon performing the first procedure in 1968\textsuperscript{26}. In the original Fontan procedure, illustrated in figure 8, the right atrium was disconnected from the rest of the heart and connected to the left pulmonary artery, thereby acting merely as a conduit for venous blood. Similarly, the inferior vena cava was connected to the right pulmonary artery.

The original Fontan operation had a high complication rate with, among other things, arrhythmias caused by a distended right atrium that also was prone to thrombus formation\textsuperscript{27}. Nowadays, patients suitable for a Fontan circulation are operated with a total cavo-pulmonary connection (TCPC) that in its latest modification uses an extracardiac conduit to connect the inferior vena cava to the pulmonary artery (figure 9).

![Diagram of Fontan circulation with TCPC](image)

**Figure 9** Fontan circulation with TCPC. Note the conduit connecting the inferior vena cava to the pulmonary artery. The connection between the superior vena cava and the pulmonary artery is referred to as a bidirectional Glenn.
**Eisenmenger syndrome**

All CHDs with unrestricted shunts of blood from the left to the right side of the heart, also those referred to as simple such as solitary atrial septal defect, patent ductus arteriosus or VSD will allow for large volumes of blood to pass through the lungs. In response to the high volumes, the pulmonary arteries may increase its vascular resistance, eventually irreversible to such a degree that the pulmonary pressure reaches systemic levels and the shunt may even reverse. At this point, it is too late to close the shunt and the Eisenmenger syndrome is established (figure 10).

![Figure 10](image)

*Figure 10 A left-to-right shunt through a VSD developing into an Eisenmenger syndrome.*

An Eisenmenger syndrome cannot be surgically corrected or palliated and is only effectively treated by a heart-lung transplant. Although the first heart transplant was performed in 1967, the first heart-lung transplant was not performed until 1981.28

Crucial for correctly diagnosing all complex CHDs mentioned above were the development of diagnostical tools such as angiography, computer tomography, echocardiography and magnetic resonance imaging.
**Adults with congenital heart disease**

Thanks to the medical and surgical success, an increasing number of children born with CHD reach adult age. As a consequence, an increasing number of patients with CHD are transferred from paediatric to adult care. At the turn of the millennium, the adult patients with congenital heart disease (ACHD) already outnumbered the children. Since the survival rates have increased mostly in complex CHD, from close to zero to approximately 85%, this group of patients grows even faster.

Adult patients with complex CHD present unique physiological conditions and medical needs to the cardiologist that include needs for interventions and complex re-intervention. Hence, these patients are referred to centres specialised in CHD. In many countries, including Sweden, these centres have been named “GUCH-centres”. GUCH stands for grown-up congenital heart disease, a term that was introduced by the British cardiologist and pioneer in the field, Jane Sommerville. But since patients nowadays are not just grown-ups, but also ageing individuals requiring life-time management in relation to their congenital condition, the European Society of Cardiology (ESC) recommends the internationally accepted ACHD in the most recent guidelines. In the said guidelines, the importance of viewing ACHD as a lifelong chronical condition is emphasised.

In general, patients with ACHD report a good quality of life, although reduced in association with functional status and level of physical exercise. However, this adult population is still young, with a mean age below 40 years. The incidences of CHD in men and women, respectively, are roughly equal, with some variations between lesions.

**Comorbidities and causes of death**

Adult patients with complex CHD are indeed survivors, but not without a cost. Cardiac comorbidities, usually related to the congenital lesion, are increased multiple times in adults with complex CHD compared to controls. Such diseases are congestive heart failure and arrhythmias that also constitute the most common causes of death in this group of patients. Other cardiac comorbidities, both directly related to the congenital lesion and acquired, are pulmonary circulation disorders and peripheral vascular disease. Adult
patients with CHD also have a high prevalence of traditional risk-factors for acquired cardiovascular disease such as hypertension and hypercholesterolemia, which increase the risk of, for instance, future coronary artery disease\textsuperscript{35-39}. It should be mentioned though that some studies suggest that patients with cyanotic lesions, interestingly, seem to have a decreased risk of coronary artery disease\textsuperscript{40}.

In addition to the cardiac comorbidities mentioned above, the risk of a number of non-cardiac comorbidities such as chronic lung disease (e.g. asthma, COPD), renal failure and neurological disorders (e.g. cerebral palsy) are approximately doubled in adults with complex CHD\textsuperscript{35,41}. The increased burden of disease, as well as risk of death at a young age, could be expressed in terms of premature ageing. This was elegantly described by Kempny et. al. as an equivalent age for different diagnoses of CHD and is illustrated in figure 11\textsuperscript{42}. As an example, a 25-year-old patient with a systemic right ventricle such as in cc-TGA has the same estimated residual lifetime as a healthy 48-year-old peer.

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Figure 11 “Equivalent age for different diagnostic groups and various ages in comparison to that observed in persons without congenital heart disease.” Reprinted with permission from Kempny et.al. "Reference Values for Exercise Limitations among Adults with Congenital Heart Disease. Relation to Activities of Daily Life–Single Centre Experience and Review of Published Data." European Heart Journal 33, no. 11 (2012): 1386-96. Here complex CHD refers to one-chamber physiology without Fontan circulation.
**Anthropometry**

Anthropometry is defined as measurements and proportions of the human body. In a Swedish register based study, men with complex CHD were shorter than reference values, as were women with Tetralogy of Fallot. Furthermore, men with Fontan physiology and pulmonary atresia had a lower body mass index (BMI) than controls, whereas no such deviations were seen in women with any type of complex CHD.

Notably, a higher than normal BMI is associated with low mortality in CHD. This phenomenon, known as the obesity paradox, is also described in congestive heart failure and coronary heart disease. The obesity paradox with an apparent protection from a high BMI is usually partly attributed to body composition with a higher skeletal muscle mass.

**Cardiorespiratory endurance**

Cardiorespiratory endurance is defined as the ability of heart and lungs to supply fuel during sustained physical activity and to eliminate fatigue. In patients with CHD, multiple factors in relation to the congenital heart defect might affect the function of the cardiorespiratory system.

**Cardiac output**

The cardiac output is the product of the stroke volume of the systemic ventricle and the heart rate.

The *stroke volume* in the systemic ventricle might be low due to multiple reasons. One example is when the filling of the systemic ventricle is affected (reduced preload), and thereby the stroke volume. This could be the case when the patient lacks a sub pulmonary ventricle such as in Fontan circulation, or when baffles are noncompliant or obstructed in patients with d-TGA operated with atrial switch. Another example is when the systolic function of the systemic ventricle is impaired. This could be the case when a morphologically right ventricle serves as a systemic ventricle, as in cc-TGA. The systolic function could also be reduced if exposed to volume overload due to a leaking valve or a shunt.

The inability to increase the *heart rate*, known as chronotropic incompetence, is common in adults with CHD. Chronotropic incompetence could be due...
to the congenital defect itself, surgery damaging the conduction system or the effect of treatment with beta blockers. Chronotropic incompetence plays an important part in reduced cardiac output in CHD, not the least in patients with a Fontan circulation with inability to increase preload and thereby the stroke volume as described above48.

Respiratory function

Not only the cardiac output, but also the respiratory system could be affected in patients with CHD. Lungs could be restrictive due to undeveloped lungs following poor blood flow in utero such as in Tetralogy of Fallot and pulmonary atresia50. Also, open heart surgery, often repeatedly, can lead to stiffness in the lungs and thoracic wall with further restriction of the lungs51.

The pulmonary blood flow can be restricted through the lungs due to a poor or non-existent sub pulmonary ventricle or obstruction of the outflow tract, or to both. Also, persistent shunts, not the least in Eisenmenger physiology, can significantly decrease the ability to oxygenate blood52.

Exercise capacity

The gold standard in assessing cardiorespiratory endurance is the cardio pulmonary exercise test (CPET) that measures the maximum rate of oxygen consumption in the body during exercise of increasing intensity – $\dot{V}O_{2}\text{max}$ 53. $\dot{V}O_{2}\text{max}$ is defined as follows:

$$\dot{V}O_{2}\text{max} = Q \times (C_a O_2 - C_v O_2)$$

Where Q is the cardiac output, $C_a O_2$ is the arterial oxygen content and $C_v O_2$ is the venous oxygen content.

In Sweden, the maximal power output during a cycle ergometer exercise test, usually measured in Watts, is often used as a more assessable indicator of exercise capacity54.

Given the multiple potential factors influencing cardiorespiratory endurance, it is not surprising that exercise capacity is low in adults with complex CHD. Furthermore, the exercise capacity, per se, independently predicts hospitalization and death42,55.
Muscle function

Muscle is a contractile tissue that produces force and motion. There are three kinds of muscle cells in the human body – the smooth muscle of, for instance, the viscera and blood vessels, the striated muscle tissue in the skeletal muscles and the striated myocardium in the heart. Throughout this thesis, when using the term “muscle”, I am referring to skeletal muscle, unless otherwise specified.

Muscle function can be measured in terms of, for instance, strength or endurance. *Muscular strength* is the maximal force that a muscle or muscle group can generate. *Muscular endurance* is the capacity to perform repeated muscle contractions. The muscle contraction can be categorized as isometric or isotonic, depending on the change in muscle length during contraction. In isometric contraction, no change in muscle length takes place. In isotonic contraction, the muscle changes length during the contraction but the tension is constant. Isotonic muscle contraction is referred to as concentric if the muscle shortens during contraction, or eccentric, if the muscle lengthens during contraction.

Studies have shown that muscle function is decreased in adults with complex CHD, both in terms of muscular strength and muscular endurance. However, when starting to work on this thesis, few studies had examined the amount of muscle, as well as other tissues, in the body in these patients – the body composition.

Beyond the heart – the body composition

The composition of the body can be studied on different levels from the molecular level – comprising hydrogen, oxygen, carbon and other elements, to the whole body level – describing the different parts such as the head, trunk and appendages. This thesis is focused on the tissue-organ level and the study of skeletal muscle and adipose tissue (papers I and III) and bone (papers II and IV).

Body composition is central in fitness sports, but also in normal ageing and disease. Following the first three decades of life, a slow decline in muscle and bone mass as well as a rise in fat mass is generally seen, even in a healthy ageing process. Not only ageing, but also both acute and chronic disease
may cause pathological changes in body composition\textsuperscript{61}. On the upside is that food and physical activity can help to build a healthy body\textsuperscript{62}. The importance of a healthy body composition cannot be stressed enough, the significance of which lies in its role in resisting the impact of stress and disease\textsuperscript{63,64}.

Prior to this thesis, there were few studies on body composition in children and young adults with Fontan physiology. Two studies reported low bone mineral density in said patients\textsuperscript{65,66} and one study reported low lean mass\textsuperscript{67}. In another study, adolescents with all forms of CHD had no decrease in bone parameters\textsuperscript{68}. No research had been done on body composition in ageing adults or in all forms of complex CHD. However, the numerous non-cardiac comorbidities, short stature, and low exercise capacity and muscle function suggested an impact of the complex congenital defects beyond the heart.

Two terms related to an unfavourable body composition are central in this thesis – sarcopenia and osteoporosis. Hence, a short description follows to facilitate for the reader.

**Sarcopenia**

Sarcopenia is defined as “a progressive and generalised skeletal muscle disorder that is associated with increased likelihood of adverse outcomes including falls, fractures, physical disability and mortality”\textsuperscript{69}. An operational definition of sarcopenia is expressed as the following criteria:

1. Low muscle strength
2. Low muscle quantity or quality
3. Low physical performance

Probable sarcopenia is identified by criterion 1, and the diagnosis is confirmed by additional documentation of criterion 2. If criteria 1, 2 and 3 are all met, sarcopenia is considered severe. Sarcopenia is usually described in the elderly and is associated with poor prognosis and death, and is then referred to as primary sarcopenia. It can also present in chronic as well as acute disease, then often referred to as secondary sarcopenia\textsuperscript{69}.

The term sarcopenia was first described in 1989\textsuperscript{70}. The definition as such has changed over time and even during the writing of this thesis. In early guidelines, the definition was based solely on decreased muscle mass,
followed by the addition of either low strength or low physical performance\textsuperscript{71,72}. Today, as described above, emphasis is shifted more towards the strength, mainly since it is easier to assess. Regardless, sarcopenia increases the risk of incident disability, hospitalization, and mortality, although with potential effective treatment in the predominant form of resistance training\textsuperscript{73-75}.

**Osteoporosis**

Osteoporosis is defined as “a progressive systemic skeletal disease characterized by low bone mass and microarchitectural deterioration of bone tissue, with a consequent increase in bone fragility and susceptibility to fracture”\textsuperscript{76}. The significance of osteoporosis lies in the fragility fractures, in Sweden estimated to affect approximately 50 \% of all women and 25 \% of all men during their lifetime\textsuperscript{77}. Ultimately, fragility fractures are a cause of pain, function loss and death, at a substantial cost for the individual and the society\textsuperscript{77}.

Bone is formed in youth and adolescence, and develops to a large extent in response to mechanical stress and resistance\textsuperscript{78}. The bones peak in mass and integrity during the third decade, then is usually slowly deteriorating throughout life\textsuperscript{79}. Primary osteoporosis is related to bone deterioration due to ageing and oestrogen deficiency in post-menopausal women. Secondary osteoporosis is related to disease, medication and lifestyle with a few examples being renal failure, glucocorticoid use and immobility, respectively\textsuperscript{80}.

Osteoporosis, in combination with other risk factors for fractures, is used to calculate the future risk of fractures and to guide further treatment. The disease can be prevented through optimal calcium intake and physical activity, not the least in youth and adolescence. Once osteoporosis manifests itself, potent pharmacological treatment exists, although these are underused\textsuperscript{81}.
Rationale

The population of adults with complex CHD is young and is continuously increasing in number.

Knowledge is sparse about the long-term effect of complex CHD on body composition. However, the presence of shorter stature, reduced muscle function and decreased exercise capacity imply an effect of the complex congenital heart defect beyond the heart. Still, at the beginning of this thesis, there was no previous studies addressing the body composition in ageing adults with various forms of complex CHD.

A pathological alteration in body composition provides important prognostic information, e.g. low muscle mass and function (sarcopenia) as well as decreased bone quality (e.g. osteoporosis) increase morbidity and mortality. Also, increased fat mass causes metabolic disorders that contribute to substantial morbidity in the general population.

If such alterations in body composition were to exist in adults with complex CHD, in addition to prognostic information, they would also provide potential targets for intervention. Interventions in form of resistance exercise training and nutrition would have a potential to reduce future morbidity and prolong life even further.
Aim

The overall aim of this thesis was to describe the body composition in adult patients with complex CHD.

More specific aims were, within an adult population of complex CHD, to describe:

1) the elements of sarcopenia, more precisely:
   - *muscle quantity* with DXA (paper I) and pQCT (paper III)
   - *muscular strength* (papers I and III)
   - *physical performance* in terms of exercise capacity (paper III)

2) elements of bone quality, in terms of:
   - *bone mass* and *bone density* with DXA (paper II) and pQCT (paper IV),
   - *bone strength* (paper IV)
   - *risk factors for reduced bone strength* (paper IV)
   - *factors associated with bone density and bone strength* (papers II and IV)

3) elements of body fat, such as:
   - *fat quantity* and (papers I and III)
   - *fat distribution* as well as *gender differences* (papers I and III).

All in comparison to an age and gender matched control group.
Materials and methods

Participants

Participants were recruited for two cross-sectional studies, hence referred to as the DXA study and the pQCT study.

The DXA study was conducted between March 2016 and November 2017. It resulted in two articles, one on sarcopenia and body fat (paper I) and one on bone quality (paper II).

The pQCT study was conducted between March 2019 and June 2019 and resulted in two articles – one on muscle mass and fat mass (paper III), and one on bone strength (paper IV).

Throughout the thesis, when reporting and discussing results, I follow the structure of (1) muscle, (2) bone and (3) fat variables. Consequently, results from all four papers are reported intermixed. This is to facilitate the understanding of the overall results regarding body composition.

Patients

Patients for both studies were identified in Swedish clinics specialized in ACHD.

Inclusion criteria were:

1) \( \geq 18 \) years of age
2) complex CHD as described in the introduction
3) clinically stable condition

There was one difference, however, between the studies regarding the inclusion criteria; although patients with d-TGA and arterial switch are considered as having complex lesions, the corrective surgery is supposed to be very effective. For instance, those patients present a better exercise capacity than any other group of complex lesions\(^2\). Hence, in the pQCT study, patients with d-TGA and arterial switch were excluded.
Remaining exclusion criteria were:

1) cognitive impairment or psychical illness affecting the independent capacity of decision making
2) genetic syndromes affecting the body composition, e.g. Downs syndrome
3) comorbidities affecting the physical function e.g. rheumatoid arthritis or severe back problems
4) ongoing pregnancy or delivery in the past three months
5) other reasons rendering them unsuitable for study participation, e.g. social circumstances or having received a heart transplant

In the DXA study, patients were recruited from the centres for ACHD in Umeå, Lund and Uppsala. In Umeå, the intention was to identify all potentially eligible patients. In Lund and Uppsala, patients potentially eligible to participate were identified by the local investigator, a so-called convenience sample.

In the pQCT study, patients were recruited from the Northern region comprising the centre in Umeå and associated clinics in Sunderbyn,
Skellefteå, Östersund and Sundsvall. Patients were primarily contacted by phone and asked for participation. The process of inclusion and exclusion in both studies is further illustrated in figure 12.

In total, 93 unique patients were included in the studies, of whom 37 % were female (n = 34). As illustrated, there was an overlap of 29 patients between the DXA study and the pQCT study. Study characteristics for the DXA study and the pQCT study as well as the resulting papers are summarized in table 1.

**Control subjects**

Control subjects were recruited in the same manner in both studies, i.e. for each patient, one control bearing the same sex and a birth date close to a corresponding patient was identified using a prespecified algorithm in the population register comprising the municipality of Umeå and was recruited by phone. Criteria of exclusion were the presence of CHD as well as the same exclusion criteria for the aforementioned patients. In total, 122 controls were included in the studies.

**Ethics**

All participants received oral and written information and gave their written informed consent to participation. The study protocols conformed to the Declaration of Helsinki and were approved in advance by the Regional Ethics Review Board (Dnr M2016-18-31M, Dnr 2016-462-32M, Dnr 2017-203-32M and Dnr 2018-529-32M).
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<td>Matching for</td>
<td>Age and sex</td>
<td>Age and sex</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: DXA, Dual-energy X-ray Absorptiometry; pQCT, peripheral Quantitative Computer Tomography; d-TGA, dextro Transposition of the Great Arteries; cc-TGA, congenitally corrected Transposition of the Great Arteries. * 29 patients overlapping.*
Participant characteristics

Basic anthropometric measurements for all participants were assessed at the study visit. Height was measured with a wall-mounted gauge and weight was measured on a digital scale. Waist and hip circumferences were measured according to the WHO data gathering protocol\textsuperscript{83}.

For patients in both studies, information regarding function of the systemic ventricle assessed with echocardiography or magnetic resonance imaging was taken from medical records from the closest clinical visit. Additionally, in the pQCT study, proBNP and s-creatinine were taken from medical records from the closest clinical visit. Creatinine based eGFR was calculated according to the revised Lund-Malmö equation as recommended\textsuperscript{84, 85}.

Imaging

*Dual-energy x-ray absorptiometry (DXA)*

DXA stands for dual energy x-ray absorptiometry and is the gold standard in assessing body composition\textsuperscript{86}.

The patient is placed on a table in the supine position. X-ray beams of two different energies are sent through the body, back to front, and measured by a detector on the opposite side. Since different tissues absorb different energies, the compositions of the transmitted x-ray beams can be used to calculate the quantity of different tissues\textsuperscript{87, 88}. DXA was first developed to assess regional bone mineral content in the spine, pelvis and femoral neck. Today, DXA is also used to assess full body content of bone, fat and lean body mass, an example of such is shown in figure 13\textsuperscript{88}.

In the DXA study, full body measurements were taken and regions of interest were manually adjusted if needed. All measurements were made with Lunar iDXA (ME-200149, 210492, 210494, 212003, General Electrics Healthcare Madison, WI, US).
Following is a brief description of the DXA-derived variables that were further analysed:

**Lean**

Lean mass (LM), sometimes referred to as just “lean”, was calculated as the total body weight minus bone and fat. LM is considered to represent skeletal muscle mass. However, LM in the trunk also represents the inner organs. Hence, appendicular lean mass (ALM), that makes up the sum of lean mass in the arms and legs, is used as a representative for skeletal muscle mass, thus excluding the bias from the inner organs.

Relative LM was calculated by dividing ALM by the height squared (ALM-index), in the same way that BMI is calculated by dividing weight by the height squared$^{72}$.

**Bone**

Bone is made up mainly of minerals, and the bone mineral content (BMC) with DXA is referred to as bone mass$^{89}$. DXA is a two-dimensional technique projecting the three-dimensional origins of data on an area. Dividing BMC
by the area of the projection provides areal bone mineral density (BMDa) in g/cm². Note that the density is two-dimensional.

*Fat*

Appendicular fat mass (AFM), whole body fat (BF) mass, and the proportion of whole body fat mass in relation to the full body weight (BF%) were calculated. The internal distribution of fat mass was described in terms of (1) the abdominal fat mass around inner organs, or ‘visceral adipose tissue’ (VAT) and (2) the proportion between android (the belly) and gynoid (hip and thigh) fat mass (FM android/gynoid).

DXA shows a high reliability and accuracy in repeated measurements. As an example, the typical error of measurement in total-body analysis for LM is 0.24 kg in females and 0.42 kg in males, for FM is 0.25 kg in females and 0.52 kg in males, and for BMC is 0.02 kg and BMD 0.01 g/cm² in both sexes. The intraclass correlation coefficients range 0.98-1.0⁹⁰.

**Peripheral quantitative computed tomography (pQCT)**

pQCT stands for peripheral quantitative computed tomography. It provides computer tomography slices of the limbs at standardized locations. In the pQCT study, both the forearm and calf were examined at a proximal and a distal site according to protocol. The proximal site was at 66 % of the bones’ length in the proximal direction. The distal site was at 4 % of the bones’ length in proximal direction close to the wrist or ankle. The two sites are exemplified by the calf in figure 1⁴⁹¹,⁹².

The non-dominant side was examined as appropriate. This was to account for an increase in muscle mass and bone mass due to handedness. Two exceptions were made. One control had had a previous fracture in the left non-dominant arm, and therefore the right dominant arm was tested instead. One control had had a previous fracture on their right non-dominant leg, and therefore the left dominant leg was tested instead.

The voxel size was 0.5 × 0.5 × 2.0 mm with a slice thickness of 2 mm. The equipment used was XCT-2000 (Stratec Medizintechnik, Pforzheim, Germany)⁹³.
Based on tissue density, software separates the compartments of muscle, bone and fat. According to the manufacturer’s algorithms, variables describing skeletal muscle and fat are measured at the proximal 66 % site since there is almost no muscle at the distal site. Likewise, the outer cortical bone is analysed at the proximal site where it is thick and nearly circular. However, the inner trabecular bone is studied at the distal 4 % site where it has a big cross-sectional area (CSA). An example picture from the two sites in the forearm is shown in figure 15.

The following variables were analysed:

**Skeletal muscle**

Muscle CSA was measured as well as muscle density. Muscle density is proposed to reflect the quality of the muscle, i.e. a high lipid content providing a low density, and a well-trained muscle rich in capillaries a higher density\(^{94}\).

**Bone**

BMC was measured at the 66 % site.
Contrary to DXA, pQCT provides bone mineral density for a volume (BMD) in mg/cm$^3$. BMD was measured in the cortex and marrow at the 66 % and 4 % sites, respectively.

Bone strength depends on density but also on architectonic parameters both on a macro- and micro-scale$^8$. For a tubular object such as a bone, resistance depends mainly on the distance of the material from the centre. The resistance to bending, in the lateral (x) and antero-posterior (y) directions, at the 66 % site can be calculated through the strength-strain index (SSI). SSI for a force working perpendicular to the x-axis is calculated as follows:

$$xSSI = \sum (x^2 \times a \frac{BMD_{vox}}{BMD_{max}}) x_{max}$$

In the formula $x = $ distance of a voxel from the central plane, $a =$ area of a cross section of a voxel, $BMD_{vox} =$ BMD in the voxel, $BMD_{max} =$ normal theoretical BMD, $x_{max} =$ maximal distance of any voxel from the central plane.

The higher the SSI, the greater the force required to fracture the bone. SSI predicts approximately 80 – 85 % of the force required to fracture the bone at the diaphysis in a bending test$^{95,96}$. 

Figure 15 pQCT of the left forearm at 4 % and 66 %. Dark grey areas represent fat and light grey areas are comprised of skeletal muscle. The red represent capillaries.
**Fat**

Finally, subcutaneous fat CSA was measured. Note that intramuscular fat as well as fat in the bone marrow do not count as fat CSA. Fat CSA was also scaled to the sum of total CSA to calculate the proportion of fat (fat CSA %). Computer derived measures of CSA, both of muscle and fat, correlate strongly \((r = 0.99)\) with MR-derived measures\(^97\).

**Muscle strength tests**

Isometric strength tests were used throughout the studies since they are easy to apply and standardize, and they correlate well with muscle mass\(^98\).

In the DXA study, isometric strength testing of hand grip, elbow flexion and knee extension were performed on the dominant side. Hand grip was chosen since it is reliable, and elbow flexion and knee extension chosen since they represent big muscle groups, which best correspond to lean mass in the arm and legs assessed with DXA\(^99,101\). In the pQCT study, as previously mentioned, imaging of the forearm and calf were performed on the non-dominant side. Therefore, isometric strength testing of hand grip and plantar flexors of the calf were also performed on the non-dominant side. In this way, the variables from the pQCT images and the variables from the strength tests represent the same muscle groups.

The investigator provided verbal guidance for when to start the contraction, encouragement during the entire contraction, and when participants should stop. The participants were allowed some familiarization with the testing equipment and were instructed to hold the contraction for five seconds. They continuously received visual feedback regarding the force achieved from the monitor of the dynamometer in all tests except for the hand grip test. For all tests, three repeated measurements were performed that were separated by one minute of rest. One minute of rest between sets has been reported as enough time for recovery in maximal strength assessments\(^102,103\). The maximum peak force was used in further analyses.

The load cells used in the present studies have been reported to be as reliable and as reproducible as a computerized isokinetic dynamometer. In a recent
report, the intraclass correlation coefficient for a corresponding equipment was 0.92\textsuperscript{101}.

**Peak isometric elbow flexion strength**

The peak isometric elbow flexion strength was assessed on the dominant side using a load cell (Ktoyo 333A-500, Ktoyo Co., Ltd., Gyeonggido, South Korea). For the test, an inelastic strap was connected between the load cell and a handle that the test person held in her/his dominant hand. The test person stood with 90 degrees flexion of the elbow and the forearm while grasping the handle. They were instructed on command to perform a maximum elbow flexion, and hold it for five seconds. See figure 16 for schematic illustration. Elbow flexion has high intra-rater and inter-rater reliability\textsuperscript{100}.

![Figure 16 Peak isometric elbow flexion strength.](image)

**Peak isometric hand grip strength**

Peak isometric strength of the forearm muscles was assessed by testing hand grip strength as illustrated in figure 17. Participants stood with shoulder adducted and elbow flexed to 90 degrees while gripping a hand-held dynamometer (SH5001 Hydraulic Hand Dynamometer, Saehan Corp., South Korea) with full force. Peak isometric hand grip strength has high inter-rater reliability as well as test-retest reliability\textsuperscript{99}.

![Figure 17 Peak isometric hand grip strength.](image)
Peak isometric knee extension strength

The peak isometric knee extension strength was assessed on the dominant side using a load cell (Ktoyo Co). For the test, an inelastic strap was connected between the load cell and a strap attached around the ankle. The test person sat on a bench with back support and with 90 degrees of knee flexion. They were instructed on command to perform a maximum knee extension and to hold the contraction for five seconds. See figure 18 for illustration. Isometric knee extension has shown a high test-retest reliability.101

Peak isometric plantar flexion strength

The peak isometric muscle force in plantar flexion of the foot was assessed using a load cell (Anyload VETEK 0-5000N; VETEK, Fioarno, Italy). Participants sat in a standardized position on a gurney with the test leg resting on the gurney with the knee in a straight position and with the angle of the ankle positioned at 90 degrees flexion. The foot of the opposite leg was positioned on the floor or on a footrest, and the knee was at approximately 90 degrees of flexion. The dynamometer was positioned resting on the knee and was attached with straps around the waist and under the foot. The foot-strap was placed over the metatarsophalangeal joint. Participants wore a cast shoe to prevent the straps from moving and a padded plank was placed behind the back to prevent the straps from being uncomfortable when performing the test. Participants were instructed on command to press the foot towards the strap (plantar flexion) as hard as possible for five seconds. The illustration is seen in figure 19.
Exercise capacity

Physical performance was assessed through standardized cycle ergometer exercise tests and was collected from medical records for patients in the pQCT study\textsuperscript{\ref{104}}. Tests were performed at a median of 1.5 years prior to inclusion in the study (IQR: -3.8 to 0.2). The maximal exercise capacity was measured in watts, compared to national reference standards, and expressed as a percentage of expected exercise capacity at time of exercise testing\textsuperscript{\ref{54}}. An exercise capacity below 75\% of expected was defined as reduced, as currently recommended by the Swedish Society of Clinical Physiology\textsuperscript{\ref{105}}.

Self-reported physical activity

*Habitual physical activity – IPAQ*

Self-reported habitual physical activity was assessed using the short version of the International Physical Activity Questionnaire (IPAQ), in both studies. The IPAQ comprises four generic items that regard time spent at different intensity levels of physical activity and a summary (vigorous, moderate, walking and total activity) in daily living during the past seven days. This is summarized as a continuous score of metabolic equivalents of task (MET) minutes/week and further divided into categories of low (<600 MET-min/week), moderate or high (≥ 3000 MET-min/week) habitual physical activity, as stated in the IPAQ scoring protocol\textsuperscript{\ref{106}}.

*Physical exercise training*

Physical exercise is the physical activity being “planned, structured, and repetitive and has as a final or an intermediate objective the improvement or maintenance of physical fitness”\textsuperscript{\ref{47}}.

In both studies, patients were asked if they performed any kind of exercise training regularly. Since *resistance training* (when the muscle is set to work against a force with the goal of increasing muscle strength and/or volume) is known to affect muscle mass more than *aerobic exercise training* (aiming at increasing cardiopulmonary endurance), in the pQCT study, both training modes were assessed separately.
Statistics

All calculations were made in IBM SPSS Statistics Version 27.0 (IBM corp., Armonk, NY, USA.).

Continuous variables were checked for normality and presented either as means with standard deviation or median with interquartile range (lower to upper). Differences in continuous variables between patients and controls were analysed with Students t-test. Ratios were presented as numbers with percentage, and differences between groups were analysed with the Chi-square test. The null hypothesis was rejected on p-values ≤ 0.05.

The effect size for Students t-test was calculated with Cohen’s d in key variables. The effect size was consider small if Cohen’s d ≥ 0.2, medium if d ≥ 0.5 and large if d ≥ 0.8 as suggested by Cohen107.

In the results section, results from the pooled study cohorts are reported if not otherwise specified. Measurements are taken from the first time of participation. In the case of any discrepancies between the studies, this is given an account.

Reference values

The calculations of full body BMD T-scores and Z-scores derived from DXA were made in relation to the NHANES/Lunar reference population108. The BMD T-score reflects the number of standard deviations from a 20–30 years old female reference population. A T-score of the femoral neck below -2.5 is defined as osteoporosis109. The BMD Z-score gives the number of standard deviations from the age and sex matched reference population.

Overweight and obesity according to anthropometric measurements was defined as BMI ≥ 25 kg/m² as recommended by WHO110. Obesity according to DXA was defined as a proportion of BF > 35 % for females and > 25 % for males according to the commonly used standard thresholds.

Isometric hand grip strength was compared to reference values from the German reference material described by Steiber111. The material was chosen since it provides reference values not only based on sex and age but also on height. In the said study, 1–1.5 SD below the mean of a sex, age and height matched control group, was associated with a hazard ratio of 1.8 of dying
within eight years. Hence, critically low grip strength was defined as below 1 SD of reference\textsuperscript{111}.

**Sarcopenia**

Lean mass within the sarcopenic range was calculated according to Newman et. al. This model also identifies individuals with a decreased muscle mass in proportion to an increased fat mass, i.e. sarcopenic obesity. The relationship between ALM and height (in meters) and fat mass (in kg) was modelled in a linear regression model for the control group, for women and men separately. The linear regression models for ALM in women was $-17.12 + 20.69 \times (\text{height}) + 0.06 \times (\text{fat mass})$ and in men was $-28.82 + 29.95 \times (\text{height}) + 0.11 \times (\text{fat mass})$. The individuals, both patients and controls, identified by the 10th percentile of the residuals of the regression, were within the sarcopenic range\textsuperscript{112}.

Lean mass within the sarcopenic range was also calculated according to Baumgartner et. al.: An ALM-index $\leq 5.45 \text{ kg/m}^2$ for women and $\leq 7.26 \text{ kg/m}^2$ for men would classify as within the sarcopenic range\textsuperscript{71}.

**Adjustment for height**

Muscle mass and strength increases with increasing height\textsuperscript{111}. Therefore, to adjust for height as a covariate when comparing means of muscle variables such as muscle CSA or strength between patients and controls, a one-way-ANCOVA was used.

Likewise, to adjust for height as a covariate when comparing means of bone variables such as BMC, BMDa and SSI as well as cortical bones inner and outer perimeters and area between patients and controls, a one-way ANCOVA was used.

The effect size for all ANCOVA is reported as partial eta squared ($\eta^2$). As a rule of thumb, the effect size can be considered small if $\eta^2 \geq 0.01$, medium if $\eta^2 \geq 0.06$ and strong if $\eta^2 \geq 0.14$. 
Correlations

Strength – muscle quantity and exercise capacity

In general, assessing muscle strength is easier than assessing muscle quantity with DXA or pQCT. Muscle strength also correlates well with muscle mass\textsuperscript{98}, which minimizes the need for measuring the latter. Hence, the correlation between isometric muscle strength and muscle quantity was tested with Pearson’s correlation in both studies.

Likewise, assessing muscle strength is easier than assessing exercise capacity. Hence, correlation between isometric strength in hand grip and exercise capacity in the pQCT study was tested with Pearson’s correlation.

Exercise training – muscle quantity

Lack of physical exercise could contribute to decreased muscle quantity and strength, and the prescription of physical activity is a useful tool to increase health in numerous diagnoses\textsuperscript{113}. To explore the relationship between self-reported participation in regular exercise training in general and resistance training in particular and muscle quantity (in terms of ALM-index or muscle CSA), the associations were tested with point biserial correlation.

The effect size of the correlation was considered small if Pearson’s $r \geq 0.1$, medium if $r \geq 0.3$ and strong if $r \geq 0.5$ as suggested by Cohen\textsuperscript{107}.

Factors associated with muscle mass and bone strength

Factors associated with muscle mass (ALM-index) and bone quality (BMDa and SSI) in patients were analysed by multivariable linear regression, with ALM-index, BMDa and tibiae SSI as dependent variables in the respective models.

Independent variables chosen due to their established or suggested connection to muscle mass were as follows: systemic ventricular function, age at intervention, arterial oxygen saturation and habitual physical activity in terms of IPAQ. Independent variables chosen, due to their established or suggested connection to bone quality and strength were as follows: weight, height, systemic ventricular function, arterial oxygen saturation, eGFR (available only in the pQCT study), loop diuretics and warfarin. The
independent variables included in the multivariable linear regression were those with \( p \)-values < 0.15 in a simple linear regression model.

Sensitivity analyses of the models were performed, stepwise excluding one independent variable at a time to ensure that the associations remained robust.

**Fontan circulation**

Patients with Fontan circulation present a bigger decrease in exercise capacity and life expectancy than most other patients with complex CHD\textsuperscript{36, 42}. Therefore, in the group of complex CHD, patients with Fontan circulation could stand for the alterations in body composition on group level. Hence, a one-way ANOVA was conducted to compare key variables from DXA and pQCT, respectively, between patients with Fontan circulation, patients with other lesions of complex CHD and controls. Key variables chosen were ALM-index from DXA and tibiae xSSI from pQCT. Tukeys HSD was used to adjust for multiple comparisons.

**Ethical considerations**

DXA and pQCT use quite low dosages of ionizing radiation, comparable to no more than one week of background radiation\textsuperscript{92}. Skeletal muscle testing might be uncomfortable and result in delayed muscle soreness but will not leave any persisting injury. On the whole, the potential benefits of the results were considered to outweigh the risks for participants.
Results

Participant characteristics

Data for all included patients are presented in table 2. For those patients occurring in both studies, data regarding the patient in this table is from the first time of assessment.

Patient and control characteristics are described in table 3. There were no differences regarding use of tobacco or habitual physical activity. As expected, patients were prescribed cardiovascular medication to a higher extent than controls. For those patients occurring in both studies, data regarding the patient and corresponding control are collected from the first time of assessment.
Table 2 Patient characteristics

<table>
<thead>
<tr>
<th></th>
<th>Patients n = 93</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>n (%)</td>
</tr>
<tr>
<td>Age, years</td>
<td>mean ± SD</td>
</tr>
<tr>
<td>Women</td>
<td>34 (37)</td>
</tr>
<tr>
<td>Age, years</td>
<td>34.0 ± 13.8</td>
</tr>
</tbody>
</table>

**Diagnosis**

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TCPC</td>
<td>29 (31)</td>
</tr>
<tr>
<td>Tetralogy of Fallot</td>
<td>26 (28)</td>
</tr>
<tr>
<td>d-TGA with atrial switch</td>
<td>18 (19)</td>
</tr>
<tr>
<td>d-TGA with arterial switch</td>
<td>6 (7)</td>
</tr>
<tr>
<td>cc-TGA</td>
<td>6 (7)</td>
</tr>
<tr>
<td>Pulmonary Atresia</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Miscellaneousa</td>
<td>5 (5)</td>
</tr>
</tbody>
</table>

**Interventions**

<table>
<thead>
<tr>
<th>Intervention</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiac intervention</td>
<td>87 (94)</td>
</tr>
<tr>
<td>Age at intervention, years</td>
<td>median (IQR)</td>
</tr>
<tr>
<td></td>
<td>2.3 (1.0 – 4.9)</td>
</tr>
</tbody>
</table>

**Functional class**

<table>
<thead>
<tr>
<th>Functional class</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NYHA I</td>
<td>59 (63)</td>
</tr>
<tr>
<td>NYHA II</td>
<td>26 (28)</td>
</tr>
<tr>
<td>NYHA III</td>
<td>5 (5)</td>
</tr>
<tr>
<td>Extracardiac limitationb</td>
<td>3 (3)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Oxygen saturation, %</th>
<th>median (IQR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>97 (94 – 98)</td>
</tr>
</tbody>
</table>

**Systemic ventricle**

<table>
<thead>
<tr>
<th>Systemic ventricle</th>
<th>n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal or slightly reduced</td>
<td>80 (86)</td>
</tr>
<tr>
<td>Moderately or severely reduced</td>
<td>13 (14)</td>
</tr>
</tbody>
</table>

Abbreviations: TCPC, total cavo-pulmonary connection; d-TGA, dextro transposition of the great arteries; cc-TGA, congenitally corrected transposition of the great arteries; NYHA, New York Heart Association functional classification. a Including sporadic cases of cc-TGA with double switch, Eisenmenger, truncus arteriosus, complete AVSD and other. b Limited by joint related problem while walking/running (n = 2), impaired balance related to previous stroke (n = 1).
Table 3 Characteristics of all included patients and controls

<table>
<thead>
<tr>
<th></th>
<th>Patients n = 93</th>
<th>Controls n = 93</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Women</td>
<td>n (%)</td>
<td>34 (37)</td>
<td>34 (37)</td>
</tr>
<tr>
<td>Age, years</td>
<td>mean ± SD</td>
<td>34.0 ± 13.8</td>
<td>34.0 ± 13.8</td>
</tr>
<tr>
<td><strong>Tobacco</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Active smoker</td>
<td>n (%)</td>
<td>5 (5)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Former smoker</td>
<td>n (%)</td>
<td>3 (3)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>User of snus</td>
<td>n (%)</td>
<td>16 (17)</td>
<td>19 (20)</td>
</tr>
<tr>
<td><strong>Cardiovascular medication</strong></td>
<td>n (%)</td>
<td>54 (58)</td>
<td>7 (8)</td>
</tr>
<tr>
<td>ARB</td>
<td>n (%)</td>
<td>8 (9)</td>
<td>4 (4)</td>
</tr>
<tr>
<td>ACE-inhibitor</td>
<td>n (%)</td>
<td>26 (28)</td>
<td>2 (2)</td>
</tr>
<tr>
<td>Beta blocker</td>
<td>n (%)</td>
<td>27 (29)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Calcium channel blocker</td>
<td>n (%)</td>
<td>4 (4)</td>
<td>3 (3)</td>
</tr>
<tr>
<td>Digoxin</td>
<td>n (%)</td>
<td>3 (3)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Diuretics</td>
<td>n (%)</td>
<td>13 (14)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>MRA</td>
<td>n (%)</td>
<td>4 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Statins</td>
<td>n (%)</td>
<td>4 (4)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>Aspirin</td>
<td>n (%)</td>
<td>17 (18)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Warfarin</td>
<td>n (%)</td>
<td>19 (20)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>NOAC</td>
<td>n (%)</td>
<td>4 (4)</td>
<td>0 (0)</td>
</tr>
<tr>
<td><strong>IPAQ</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lowa</td>
<td>n (%)</td>
<td>14 (15)</td>
<td>9 (10)</td>
</tr>
<tr>
<td>Moderate</td>
<td>n (%)</td>
<td>49 (53)</td>
<td>50 (54)</td>
</tr>
<tr>
<td>High</td>
<td>n (%)</td>
<td>30 (32)</td>
<td>34 (37)</td>
</tr>
</tbody>
</table>

Abbreviations: ARB, angiotensin II receptor blockers; ACE-inhibitor, angiotensin converting enzyme – inhibitor; MRA, mineralocorticoid receptor agonist; NOAC, new oral anticoagulant; IPAQ, international physical activity questionnaire. a Low < 600 MET-min/week, moderate ≥ 600 and < 3000 MET-min/week and high ≥ 3000 MET-min/week.
**Anthropometry**

Basic anthropometric measures for participants are described in table 4. Both female and male patients were shorter than controls but with no differences regarding BMI. Female patients had a wider waist but not a wider hip and hence an increased waist/hip-ratio compared to the control group.

**Table 4 Anthropometry**

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th></th>
<th>Men</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients (n = 34)</td>
<td>Controls (n = 34)</td>
<td>p</td>
<td>Patients (n = 59)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>162.5 ± 7.6</td>
<td>166.9 ± 5.6</td>
<td><strong>0.01</strong></td>
<td>177.1 ± 6.7</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>65.7 ± 11.5</td>
<td>65.1 ± 8.4</td>
<td>0.8</td>
<td>75.5 ± 10.9</td>
</tr>
<tr>
<td>BMI kg/m²</td>
<td>24.8 ± 3.7</td>
<td>23.4 ± 2.6</td>
<td>0.7</td>
<td>24.0 ± 2.9</td>
</tr>
<tr>
<td>Waist, cm</td>
<td>84.1 ± 11.0</td>
<td>77.0 ± 7.4</td>
<td><strong>0.003</strong></td>
<td>89.2 ± 9.9</td>
</tr>
<tr>
<td>Hip, cm</td>
<td>99.8 ± 10.5</td>
<td>96.6 ± 7.3</td>
<td>0.1</td>
<td>96.3 ± 6.3</td>
</tr>
<tr>
<td>Waist/Hip</td>
<td>0.84 ± 0.08</td>
<td>0.80 ± 0.05</td>
<td><strong>0.008</strong></td>
<td>0.93 ± 0.07</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD. Abbreviations: BMI, body mass index. For those patients occurring in both studies, data regarding the patient and corresponding control are collected from the first time of assessment.

The prevalence of overweight and obesity according to BMI (≥ 25 kg/m²) was equal in female patients compared to controls (41 % (n = 14) vs. 24 % (n = 8), p = 0.1) as well as in male patients compared to controls (31% (n = 18) vs. 48 % (n = 28), p = 0.06).

These pooled results regarding anthropometric variables differ between the separate studies in two ways. First, in the DXA study, female patients did present a slightly higher BMI than controls (25.7 ± 4.2 vs. 23.0 ± 2.5 kg/m², p = 0.02). Second, in the pQCT study, male patients were not shorter than controls (176.9 ± 8.0 vs. 179.9 ± 7.0 cm, p = 0.2) mainly due to a slightly shorter control group.
Skeletal muscle

Skeletal muscle quantity

Skeletal muscle quantity assessed with DXA is presented in table 5. Both female and male patients had lower lean mass in the arms, legs and full body compared to controls. ALM-index, i.e. appendicular lean mass in relation to height squared, was lower in men but not in women compared to controls. Cohen’s $d$ for the difference in ALM-index between male patients and controls were 0.95, considered a strong effect size.

Table 5 Muscle quantity with DXA

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th></th>
<th></th>
<th>Men</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients n = 22</td>
<td>Controls n = 22</td>
<td>$p$</td>
<td>Patients n = 51</td>
<td>Controls n = 51</td>
<td>$p$</td>
</tr>
<tr>
<td>Appendicular</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LM arms, kg</td>
<td>3.8 ± 0.7</td>
<td>4.4 ± 0.6</td>
<td><strong>0.01</strong></td>
<td>6.6 ± 1.2</td>
<td>7.6 ± 1.3</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>LM legs, kg</td>
<td>12.9 ± 2.2</td>
<td>14.2 ± 1.5</td>
<td><strong>0.03</strong></td>
<td>17.2 ± 2.6</td>
<td>20.1 ± 2.5</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>ALM, kg</td>
<td>16.7 ± 2.8</td>
<td>18.6 ± 2.0</td>
<td><strong>0.02</strong></td>
<td>23.8 ± 3.6</td>
<td>27.7 ± 3.6</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>ALM-index, kg/m$^2$</td>
<td>6.30 ± 0.75</td>
<td>6.67 ± 0.55</td>
<td><strong>0.06</strong></td>
<td>7.57 ± 0.97</td>
<td>8.46 ± 0.90</td>
<td><strong>&lt;0.001</strong></td>
</tr>
<tr>
<td>Full body</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>LM, kg</td>
<td>38.2 ± 4.4</td>
<td>41.6 ± 3.9</td>
<td><strong>0.01</strong></td>
<td>52.6 ± 6.0</td>
<td>58.3 ± 6.3</td>
<td><strong>&lt;0.001</strong></td>
</tr>
</tbody>
</table>

Data are presented as means ± SD. Abbreviations: LM, lean mass; ALM, appendicular lean mass.

Skeletal muscle quantity assessed with pQCT is presented in table 6. In female patients, the muscle CSA was not lower in the forearm but was lower in the calf compared to controls. In male patients the muscle CSA was lower in the forearm and calf compared to controls. There were no differences in muscle density between patients and controls, in either sex.
## Table 6 Muscle quantity with pQCT

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th></th>
<th></th>
<th>Men</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients n = 23</td>
<td>Controls n = 23</td>
<td>p</td>
<td>Patients n = 26</td>
<td>Controls n = 26</td>
<td>p</td>
</tr>
<tr>
<td>Forearm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle CSA, cm²</td>
<td>24.4 ± 2.6</td>
<td>26.9 ± 3.3</td>
<td>0.07</td>
<td>35.6 ± 5.7</td>
<td>42.9 ± 7.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Muscle density, mg/cm³</td>
<td>75.9 ± 1.6</td>
<td>76.3 ± 2.2</td>
<td>0.4</td>
<td>76.5 ± 2.1</td>
<td>76.4 ± 3.1</td>
<td>0.9</td>
</tr>
<tr>
<td>Calf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle CSA, cm²</td>
<td>59.5 ± 6.4</td>
<td>64.7 ± 5.9</td>
<td>0.03</td>
<td>69.4 ± 9.1</td>
<td>80.9 ± 8.7</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Muscle density, mg/cm³</td>
<td>74.7 ± 2.3</td>
<td>2.2 ± 73.2</td>
<td>0.7</td>
<td>73.2 ± 3.6</td>
<td>73.6 ± 3.4</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD. Abbreviations: CSA, cross-sectional area.

A one-way ANCOVA was conducted to compare muscle CSA in the forearm and calf between patients and controls, whilst adjusting for height.

In female patients, there was a difference in the mean muscle CSA in the forearm \[F(1,43) = 4.265, p = 0.05, η² = 0.09\] but not in mean muscle CSA in the calf \[F(1,43) = 1.179, p = 0.3, η² = 0.03\] compared to controls, whilst adjusting for height. Since female patients were shorter but not lighter than controls, and the additional weight on the calf muscles ought to increase the muscle mass, a post-hoc analysis adjusting for weight instead of height was performed. In the said analysis muscle CSA in the calf was lower compared to controls \[F(1,43) = 5.114, p = 0.03, η² = 0.11\]. The effect size was medium.

In male patients, there was a difference in both the mean muscle CSA in the forearm \[F(1,49) = 13.245, p = 0.001, η² = 0.21\] as well as mean muscle CSA in the calf \[F(1,49) = 17.725, p < 0.001, η² = 0.27\], whilst adjusting for height. The effect size was high.

### Sarcopenic range

Fifty-nine percent \(n = 13\) of the female patients had an ALM within the sarcopenic range compared to 9 % \(n = 2\) in the control group \(p < 0.001\). Forty-seven percent \(n = 24\) of the male patients had an ALM within the sarcopenic range compared to 10 % \(n = 4\) in the control group \(p < 0.001\).
In total, 51% of the patients (n = 37) had an ALM within the sarcopenic range.

Using the alternative definition by Baumgartner, 14% (n = 3) of the female patients had an ALM-index below the sarcopenic limit (≤ 5.45 kg/m²) compared to none in the control group (p = 0.07). Among the male patients, 47% (n = 24) were below the sarcopenic limit (≤ 7.26 kg/m²) compared to 6% (n = 3) in the control group (p < 0.001).

*Fontan circulation and other diagnoses of complex CHD*

There was a difference in the ALM-index [F(2,143) = 13.048, p < 0.001, \( \eta^2 = 0.15 \)] between patients with a Fontan circulation, patients with other diagnoses of complex CHD and controls. *Post hoc* tests showed patients with Fontan circulation had an ALM-index of -1.35 ± 0.27 kg/m² (mean ± SE) compared to controls (p < 0.001). Patients with other complex diagnoses of CHD had and ALM-index of -0.49 ± 0.20 kg/m² compared to controls (p = 0.04).

*Factors associated with muscle mass*

To identify factors associated with ALM-index in patients, variables with p < 0.15 in a simple linear regression were included in a multivariable linear regression model. Of the prespecified variables, the variables *not* significant in a simple linear regression were systemic ventricular function (p = 0.6) and age at intervention (p = 0.3). The variables that *were* significant in a simple linear regression and included in the model were: arterial oxygen saturation (p = 0.04) and IPAQ (p = 0.03). A significant regression equation was found, but the association was weak and none of the variables were significant: [F(2,70) = 3.832, p = 0.03], with an R² of 0.073. The patients’ ALM-index is equal to 1.769 + 8.3 \times 10^{-5} \times (IPAQ) + 0.05 \times (saturation), where IPAQ is coded or measured as MET-min/week and arterial oxygen saturation in %. ALM-index increased 8.3 \times 10^{-5} kg/m² for each MET-min/week and 0.05 kg/m² for every percent in oxygen saturation. In the model, neither IPAQ nor saturation were associated with ALM-index.
**Isometric muscle strength**

Results from the isometric muscle strength tests from the DXA study are presented in table 7. Both female and male patients had lower peak isometric muscular strength in elbow flexion, hand grip and knee extension on their dominant side compared to the controls.

Table 7 Peak isometric muscle strength on the dominant side in the DXA study

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients n = 22 Controls n = 22</td>
<td>Patients n = 51 Controls n = 51</td>
</tr>
<tr>
<td><strong>Arm</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elbow flexion, N</td>
<td>119 ± 24</td>
<td>142 ± 22</td>
</tr>
<tr>
<td>Hand grip, kg</td>
<td>31 ± 7</td>
<td>38 ± 6</td>
</tr>
<tr>
<td><strong>Leg</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Knee extension, N</td>
<td>310 ± 70</td>
<td>387 ± 103</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD.

A one-way ANCOVA was conducted to compare the peak isometric muscle strength described above, between patients and controls, whilst adjusting for height. All results remained robust with a medium to strong effect size as follows:

In female patients, the peak isometric strength was lower in **elbow flexion** \[F(1,41) = 8.888, \ p = 0.005, \ \eta^2 = 0.18\], **hand grip** \[F(1,41) = 10.443, \ p = 0.002, \ \eta^2 = 0.20\] and **knee extension** compared to controls \[F(1,41) = 6.613, \ p = 0.01, \ \eta^2 = 0.14\], whilst adjusting for height.

Also in male patients, the peak isometric strength was lower in **elbow flexion** \[F(1,99) = 7.570, \ p = 0.007, \ \eta^2 = 0.07\], **hand grip** \[F(1,99) = 5.772, \ p = 0.02, \ \eta^2 = 0.06\] and **knee extension** compared to controls \[F(1,99) = 5.582, \ p = 0.02, \ \eta^2 = 0.05\], whilst adjusting for height.

Results from the peak isometric muscle strength tests from the pQCT study are presented in table 8. Both female and male patients had lower peak isometric muscular strength in both hand grip and plantar flexion in their non-dominant side compared to controls.
Table 8 Peak isometric strength on the non-dominant side in the pQCT study

<table>
<thead>
<tr>
<th></th>
<th>Women Patients n = 23</th>
<th>Controls n = 23</th>
<th>p</th>
<th>Men Patients n = 26</th>
<th>Controls n = 26</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Forearm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hand grip, kg</td>
<td>29 ± 4</td>
<td>34 ± 5</td>
<td>0.001</td>
<td>46 ± 10</td>
<td>54 ± 11</td>
<td>0.008</td>
</tr>
<tr>
<td>Calf</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Plantar flexion, N</td>
<td>482 ± 151</td>
<td>619 ± 153</td>
<td>0.004</td>
<td>577 ± 215</td>
<td>708 ± 234</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Data are presented as means ± SD.

A one-way ANCOVA was conducted to compare the peak isometric muscle strength described above, between patients and controls, whilst controlling for height. The differences remained robust in female patients in both tests and in male patients in hand grip, with a medium effect size. The difference between male patients and controls in peak isometric strength in plantar flexion did not persist:

In female patients, the mean peak isometric strength was lower in hand grip [F(1,43) = 5.125, p = 0.03, η² = 0.11] and plantar flexion [F(1,43) = 6.016, p = 0.02, η² = 0.12] compared to controls, whilst adjusting for height.

In male patients, the mean peak isometric strength was lower in hand grip [F(1,49) = 4.998, p = 0.03, η² = 0.09] but not in plantar flexion [F(1,49) = 2.770, p = 0.1, η² = 0.05] compared to controls, whilst adjusting for height.

**Critically low isometric peak hand grip strength**

Among patients in the DXA study, 19 % (n = 14) had a critically low isometric peak hand grip strength compared to 4 % (n = 3) in the control group (p = 0.005) in comparison to the reference material provided by Steiber.[111]

**Correlation isometric strength and muscle quantity**

In the DXA study, the participants had a strong positive correlation between strength in isometric elbow flexion and lean mass in the dominant arm,
\( r(144) = 0.89, p < 0.001 \). Likewise, participants had a strong positive correlation between peak isometric hand grip strength and lean mass in the dominant arm, \( r(144) = 0.83, p = <0.001 \); the correlation is illustrated in figure 20.

![Figure 20](image)

Figure 20 Correlation between lean mass assessed with DXA in the dominant arm and peak isometric grip strength in the corresponding hand. Red dots are individuals with a critically low hand grip strength.

Also, in the dominant leg, participants had a strong correlation between isometric strength of knee extension and lean mass, \( r(144) = 0.67, p < 0.001 \).

In the pQCT study, in the non-dominant arm, participants had a strong positive correlation between isometric grip strength and forearm muscle CSA, \( r(96) = 0.84, p < 0.001 \).

In the non-dominant leg, participants had a moderate correlation between isometric strength in the plantar flexors and calf muscle CSA, \( r(96) = 0.32, p = 0.001 \).
Exercise training

Self-reported weekly participation in any form of exercise training was less frequent in female patients compared to controls in the DXA study (36 % (n = 8) vs. 68 % (n = 15), p = 0.04). No difference was seen between male patients and controls (45 % (n = 23) vs. 61 % (n = 31), p = 0.1).

Self-reported weekly participation in aerobic exercise training and resistance training in the pQCT study, are presented in table 9. Female patients reported participation in resistance training to a lesser extent than controls. No other differences were found regarding exercise training habits.

Table 9 Self-reported participation in exercise training in the pQCT study

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th></th>
<th>Men</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients</td>
<td>n = 23</td>
<td>Controls</td>
<td>n = 23</td>
</tr>
<tr>
<td>Aerobic exercise training</td>
<td>10 (43)</td>
<td>16 (70)</td>
<td>0.07</td>
<td>12 (46)</td>
</tr>
<tr>
<td>Resistance training</td>
<td>5 (22)</td>
<td>14 (61)</td>
<td><strong>0.007</strong></td>
<td>9 (35)</td>
</tr>
</tbody>
</table>

Data are presented as n (%).

Exercise training and muscle quantity

Analysing the correlation between self-reported exercise training and measurements of muscle quantity revealed no associations, with one exception – male patients showed a moderate association between regular exercise training and ALM-index. Detailed results are described below.

Exercise training and ALM-index with DXA

A point-biserial correlation was run to determine the association between weekly participation in any form of exercise training and ALM-index in patients in the DXA study. In female patients, there was no association between resistance training and ALM-index, whereas in male patients there was an association, although negative:
Female patients, association between regular exercise training and ALM-index: ($r_{pb} = 0.019, n = 22, p = 0.932$).

Male patients, association between regular exercise training and ALM-index: ($r_{pb} = -0.35, n = 51, p = 0.01$).

No associations were seen in the corresponding tests in controls (data not shown).

**Resistance training and muscle CSA with pQCT**

A point-biserial correlation was run to determine the association between weekly participation in resistance training and muscle CSA in the forearm in patients in the pQCT study. There was no association between resistance training and muscle CSA in either female or male patients, as follows:

Female patients, association between resistance training and muscle CSA, in the forearm: ($r_{pb} = 0.28, n = 23, p = 0.2$), in the calf ($r_{pb} = 0.25, n = 23, p = 0.3$).

Male patients, association between resistance training and muscle CSA in the forearm ($r_{pb} = -0.04, n = 26, p = 0.8$), in the calf, ($r_{pb} = 0.026, n = 26, p = 0.9$).

No associations were seen in the corresponding tests in controls (data not shown).

**Exercise capacity**

Data on maximal exercise capacity during standardized cycle ergometer exercise tests were available for 46 patients in the pQCT study. The average exercise capacity was $73 \pm 17$ % of expected, and $54 \% (n = 25)$ had an exercise capacity below $75 \%$ of expected.

There was as strong correlation between peak isometric grip strength of the non-dominant hand and exercise capacity in watts in patients, $r(44) = 0.66$, $p < 0.001$. 
Sarcopenia

In the DXA study, as stated above, 51 % (n = 37) of the patients had a muscle mass within the sarcopenic range and 19 % (n = 14) had a critically low isometric hand grip strength. Fifteen percent (n = 11) of the patients were sarcopenic, i.e. had both low lean mass and low grip strength, as compared to none among the controls.

Of the 29 patients overlapping between the DXA study and the pQCT study, 28 had performed a test for exercise capacity. Of these 28 patients, 52 % (n = 15) had a reduced muscle mass and 25 % (n = 7) had a reduced grip strength in the DXA study and 52 % (n = 15) had a reduced exercise capacity in the pQCT study. The overlapping traits are further illustrated in figure 21.

![Overlap between patients with reduced muscle mass, reduced grip strength and reduced exercise capacity in patients overlapping between the DXA study and the pQCT study. Note that information regarding muscle mass and grip strength are from the DXA study and information about exercise capacity is from the pQCT study.](image)
Bone

Risk factors for osteoporosis

Risk factors for osteoporosis in patients and controls in the pQCT study are presented in table 10. The only differences between patients and controls, regarding traditional risk factors and drugs associated with osteoporosis were the usages of loop diuretics and warfarin.

Table 10 Traditional and potential risk factors for osteoporosis in the pQCT study

<table>
<thead>
<tr>
<th></th>
<th>Patients n = 49</th>
<th>Controls n = 49</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Drugs</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corticosteroids¹</td>
<td>4 (8)</td>
<td>1 (2)</td>
<td>0.2</td>
</tr>
<tr>
<td>Loop diuretics</td>
<td>5 (10)</td>
<td>0 (0)</td>
<td>0.02</td>
</tr>
<tr>
<td>Warfarin</td>
<td>7 (14)</td>
<td>0 (0)</td>
<td>0.006</td>
</tr>
<tr>
<td>PPI</td>
<td>1 (2)</td>
<td>1 (2)</td>
<td>1.0</td>
</tr>
<tr>
<td>Levothyroxine</td>
<td>3 (6)</td>
<td>1 (2)</td>
<td>0.3</td>
</tr>
<tr>
<td>OCP</td>
<td>2 (4)</td>
<td>3 (6)</td>
<td>0.6</td>
</tr>
<tr>
<td>Anticonvulsants</td>
<td>2 (4)</td>
<td>1 (2)</td>
<td>0.6</td>
</tr>
<tr>
<td><strong>Previous osteoporotic fracture</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>In participant</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>In participants’ parent</td>
<td>9 (18)</td>
<td>5 (10)</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Alcohol and tobacco</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt; 3 units/day</td>
<td>2 (4)</td>
<td>0 (0)</td>
<td>0.2</td>
</tr>
<tr>
<td>Smoker</td>
<td>2 (4)</td>
<td>3 (6)</td>
<td>0.6</td>
</tr>
</tbody>
</table>

Data are presented as n (%). Abbreviations: PPI, proton pump inhibitor; OCP, oral contraceptive. ¹Corticosteroide usage for > 3 months.

No participant was currently prescribed heparin or lithium. No participant had rheumatoid arthritis, since this was an exclusion criterion for the study. No participant had any known secondary osteoporosis at inclusion or was underweight (BMI ≤ 18.5).
**Bone mass, bone density and bone strength**

Results from the DXA study regarding bone mass and bone density are presented in table 1. Throughout patients had lower values in all variables describing bone mass and bone density than controls. Cohen’s d for the difference in total BMDa between patients and controls was 0.69, i.e. a medium effect size.

*Table 11 Bone variables with DXA from the DXA study*

<table>
<thead>
<tr>
<th></th>
<th>Patients</th>
<th>Controls</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 73</td>
<td>n = 73</td>
<td></td>
</tr>
<tr>
<td><strong>Bone mass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arms BMC, kg</td>
<td>0.37 ± 0.10</td>
<td>0.41 ± 0.10</td>
<td>0.008</td>
</tr>
<tr>
<td>Trunk BMC, kg</td>
<td>0.78 ± 0.17</td>
<td>0.85 ± 0.17</td>
<td>0.02</td>
</tr>
<tr>
<td>Legs BMC, kg</td>
<td>0.95 ± 0.20</td>
<td>1.11 ± 0.22</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Total BMC, kg</td>
<td>2.62 ± 0.49</td>
<td>2.92 ± 0.48</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Bone density</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total BMDa, kg/m²</td>
<td>1.18 ± 0.12</td>
<td>1.26 ± 0.11</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMDa T-scorea</td>
<td>0.1 ± 1.1</td>
<td>0.9 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMDa Z-score</td>
<td>0.2 ± 1.0</td>
<td>0.9 ± 1.0</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Abbreviations: BMC, bone mineral content; BMDa, bone mineral density. a T-score for 71 patients and 70 controls (since T-score requires ≥20 years of age).

Results from the pQCT study regarding bone mass and bone density are presented in table 12. Patients had lower bone mass compared to controls in both the radius and tibia. However, no differences were found in any variables regarding bone density. Bone strength, in terms of SSI, was low in three out of four variables in the patients.
### Table 12 Bone variables with pQCT from the pQCT study

<table>
<thead>
<tr>
<th></th>
<th>Patients n = 49</th>
<th>Controls n = 49</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Radius</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bone mass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC&lt;sup&gt;a&lt;/sup&gt;, g</td>
<td>1.08 ± 0.19</td>
<td>1.17 ± 0.2</td>
<td>0.03</td>
</tr>
<tr>
<td><strong>Bone density</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trabecular BMD, mg/cm³</td>
<td>199 ± 46</td>
<td>208 ± 45</td>
<td>0.3</td>
</tr>
<tr>
<td>Cortical BMD&lt;sup&gt;a&lt;/sup&gt;, mg/cm³</td>
<td>1160 ± 29</td>
<td>1151 ± 28</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Bone strength</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>xSSI&lt;sub&gt;a&lt;/sub&gt;, mm³</td>
<td>154 ± 46</td>
<td>175 ± 54</td>
<td>0.04</td>
</tr>
<tr>
<td>ySSI&lt;sub&gt;a&lt;/sub&gt;, mm³</td>
<td>178 ± 58</td>
<td>195 ± 55</td>
<td>0.1</td>
</tr>
<tr>
<td><strong>Tibia</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bone mass</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BMC&lt;sup&gt;a&lt;/sup&gt;, g</td>
<td>3.75 ± 0.63</td>
<td>4.37 ± 0.77</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td><strong>Bone density</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trabecular BMD, mg/cm³</td>
<td>235 ± 39</td>
<td>250 ± 44</td>
<td>0.08</td>
</tr>
<tr>
<td>Cortical BMD&lt;sup&gt;a&lt;/sup&gt;, mg/cm³</td>
<td>1135 ± 25</td>
<td>1131 ± 26</td>
<td>0.4</td>
</tr>
<tr>
<td><strong>Bone strength</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>xSSI&lt;sub&gt;a&lt;/sub&gt;, mm³</td>
<td>1492 ± 399</td>
<td>1780 ± 372</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ySSI&lt;sub&gt;a&lt;/sub&gt;, mm³</td>
<td>1065 ± 304</td>
<td>1250 ± 281</td>
<td>0.002</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Abbreviations: BMC, bone mineral content; BMD, bone mineral density; SSI, strength-strain index. <sup>a</sup>Variables were measured at the 66% site.

**Fontan circulation and other diagnoses of complex CHD**

There was a difference in the tibiae xSSI [F(2,95) = 7.085, p = 0.001, η² = 0.13] between patients with a Fontan circulation, patients with other diagnoses of complex CHD and controls. *Post hoc* tests showed that patients with Fontan circulation had a tibiae xSSI of -346 ± 111 mm² (mean ± SE) compared to controls (p < 0.007). Also, patients with other complex
diagnoses of CHD had a tibia xSSI of -261 ± 87 mm² compared to controls (p = 0.01).

**Bone variables adjusted for height**

**DXA**

A one-way ANCOVA was conducted to compare total BMC and BMDa from DXA between patients and controls, whilst adjusting for height. There was a difference in mean BMC \[ F(1,143) = 9.249, p = 0.003, \eta^2 = 0.06 \] as well as in mean BMDa \[ F(1,143) = 12.392, p = 0.001, \eta^2 = 0.08 \] between groups whilst adjusting for height. Corresponding effect sizes were medium.

**pQCT**

A one-way ANCOVA was conducted to compare radii and tibiae BMC, xSSI and ySSI from pQCT between patients and controls, whilst adjusting for height.

In the **radius**, there were no differences between the groups in BMC \[ F(1,95) = 1.102, p = 0.3, \eta^2 = 0.01 \], xSSI \[ F(1,95) = 0.902, p = 0.3, \eta^2 = 0.009 \] or ySSI \[ F(1,95) = 0.031, p = 0.9, \eta^2 = 0.000 \], whilst adjusting for height.

On the contrary, in the **tibia**, differences between the groups persisted in both BMC \[ F(1,95) = 18.765, p < 0.001, \eta^2 = 0.2 \], xSSI \[ F(1,95) = 9.353, p = 0.03, \eta^2 = 0.09 \] and ySSI \[ F(1,95) = 4.890, p = 0.03, \eta^2 = 0.05 \], whilst adjusting for height. Corresponding effect sizes were small to medium. Further analyses of the tibia revealed that the periosteal (outer) perimeters were smaller \( F(1,95) = 4.520, p = 0.04, \eta^2 = 0.05 \) and the endosteal (inner) perimeters were equal \( F(1,95) = 0.005, p = 1.0, \eta^2 = 0.00 \) resulting in a smaller cortical area \( F(1,95) = 17.804, p < 0.001, \eta^2 = 0.16 \), in patients compared to controls, despite adjusting for height.

**Factors associated with bone quality**

To identify factors associated with full body BMDa in patients in the DXA study, variables significant in a simple linear regression were included in a multiple linear regression model. The variables *not* significant in a simple
linear regression, were systemic ventricular function \((p = 1.0)\) and warfarin \((p = 0.2)\). The following variables were significant in a simple linear regression and were included in the model: weight \((p < 0.001)\), height, arterial oxygen saturation \((p = 0.13)\) \((p < 0.001)\) and loop-diuretics \((p = 0.03)\). A significant regression equation was found \([F(4,68) = 7.374, p < 0.001]\), with an \(R^2\) of 0.262. The patients’ full body BMDa is equal to \(-0.035 + 0.003 \times \text{(height)} + 0.003 \times \text{(weight)} - 0.058 \times \text{(loop-diuretics)} + 0.005 \times \text{(saturation)}\), where height is coded or measured as cm, weight as kg and arterial oxygen saturation in %. Full body BMDa increased 0.003 \(kg/m^2\) for each cm and 0.003 \(kg/m^2\) for every kg, decreased 0.058 \(kg/m^2\) if loop-diuretics was used and increased 0.005 \(kg/m^2\) with every % arterial oxygen. In the model, weight and height were associated with full body BMDa but the use of loop-diuretics and arterial oxygen saturation were not. In a sensitivity analysis, excluding one factor at a time, excluding loop diuretics rendered weight nonsignificant \((p = 0.07)\) and excluding weight rendered loop diuretics nonsignificant \((p = 0.1)\).

To identify factors associated with tibiae xSSI in patients in the pQCT study, variables significant in a simple linear regression were included in a multiple linear regression model. The following variables were not significant in a simple linear regression: systemic ventricular function \((p = 0.5)\), arterial oxygen saturation \((p = 0.6)\), eGFR \((p = 0.6)\), loop diuretics \((p = 0.9)\) and warfarin \((p = 0.8)\). The variables included in the model were: height \((p < 0.001)\) and weight \((p = 0.007)\). A significant regression equation was found \([F(2,46) = 39.585, p < 0.001]\), with an \(R^2\) of 0.617. The patients’ tibiae xSSI was equal to \(-4155.8 + 35.7 \times \text{(height)} - 5.6 \times \text{(weight)}\), where height is coded or measured as cm and weight as kg. Tibiae xSSI increased 35.7 \(mm^3\) for each cm and decreased 5.6 \(mm^3\) for every kg. In the model, height, but not weight, was associated with tibiae xSSI.

When modelling the same factors with tibiae ySSI in patients, results remained robust (data not shown).
Fat quantity and distribution

The variables for fat mass and distribution in the DXA study are presented in table 13. Female patients had lower BF, both in absolute and relative numbers, compared to controls. The excess fat was distributed towards the belly; the AFM was the same contrary to the VAT and android/gynoid FM that was higher compared to controls. Cohen’s d for the mean difference in BF% between female patients and controls was 1.21, i.e. a strong effect size.

Male patients had no differences in any variables regarding fat compared to controls.

Table 13 Fat mass and distribution with DXA

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th></th>
<th></th>
<th></th>
<th>Men</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients n = 22</td>
<td>Controls n = 22</td>
<td>p</td>
<td>Patients n = 51</td>
<td>Controls n = 51</td>
<td>p</td>
<td></td>
</tr>
<tr>
<td>Appendicular</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AFM, kg</td>
<td>12.1 ± 4.4</td>
<td>9.9 ± 3.1</td>
<td>0.06</td>
<td>7.6 ± 3.0</td>
<td>7.9 ± 3.4</td>
<td>0.7</td>
<td></td>
</tr>
<tr>
<td>Distribution</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VAT-mass, g</td>
<td>805 ± 486</td>
<td>274 ± 248</td>
<td>&lt; 0.001</td>
<td>1033 ± 701</td>
<td>1011 ± 1037</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>FM android/gynoid</td>
<td>0.52 ± 0.13</td>
<td>0.34 ± 0.13</td>
<td>&lt; 0.001</td>
<td>0.63 ± 0.18</td>
<td>0.59 ± 0.24</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BF, kg</td>
<td>27.5 ± 9.4</td>
<td>20.0 ± 6.4</td>
<td>0.004</td>
<td>20.9 ± 7.6</td>
<td>20.8 ± 9.6</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>BF%, %</td>
<td>39.5 ± 7.5</td>
<td>30.8 ± 6.9</td>
<td>&lt; 0.001</td>
<td>26.9 ± 6.9</td>
<td>24.4 ± 8.42</td>
<td>0.1</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Abbreviations: AFM, appendicular fat mass; VAT, visceral adipose tissue; FM, fat mass; BF, body fat; BF%, body fat percentage.

In the DXA study, based on BF% female patients had a higher incidence of obesity than controls (78 % (n = 17) vs. 27 % (n = 6), p = 0.008), whereas male patients did not (63 % (n = 32) vs. 53 % (n = 27), p = 0.3). Female patients did not have a higher incidence of obesity compared to male patients (78 % (n = 17) vs. 63 % (n = 32), p = 0.2).

Variables for fat mass and proportion in the limbs measured with pQCT are presented in table 14. There were no differences in any variables between patients and controls in either sex.
Table 14 Fat area and distribution with pQCT

<table>
<thead>
<tr>
<th></th>
<th>Women</th>
<th></th>
<th>Men</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients n = 23</td>
<td>Controls n = 23</td>
<td>Patients n = 26</td>
<td>Controls n = 26</td>
</tr>
<tr>
<td>Forearm</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat CSA, cm²</td>
<td>18.0 ± 8.3</td>
<td>15.7 ± 6.0</td>
<td>10.6 ± 4.8</td>
<td>11.2 ± 5.4</td>
</tr>
<tr>
<td>Fat CSA%, %</td>
<td>39 ± 10</td>
<td>34 ± 9</td>
<td>21 ± 8</td>
<td>19 ± 7</td>
</tr>
<tr>
<td></td>
<td>p 0.3</td>
<td>0.1</td>
<td>0.7</td>
<td>0.3</td>
</tr>
<tr>
<td>Calf</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fat CSA, cm²</td>
<td>33.2 ± 12.4</td>
<td>30.5 ± 7.9</td>
<td>18.4 ± 7.8</td>
<td>21.9 ± 9.5</td>
</tr>
<tr>
<td>Fat CSA%, %</td>
<td>33 ± 7</td>
<td>30 ± 6</td>
<td>19 ± 7</td>
<td>19 ± 6</td>
</tr>
<tr>
<td></td>
<td>p 0.7</td>
<td>0.1</td>
<td>0.9</td>
<td></td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD. Abbreviation: CSA, cross sectional area.
Discussion

In an adult population of patients with complex CHD, reduced muscle mass, muscle strength and physical function was found – all traits of sarcopenia. Patients also had decreased bone mass and bone strength, especially in the legs. Female patients had increased amount of fat mass, predominantly distributed around the internal organs.

Methodological considerations

Methodological discussion will be presented using some key concepts in methodology, and pointing towards strengths as well as acknowledging limitations.

Key concepts in methodology

Reliability refers to precision or whether an instrument produces stable, consistent and reliable results without random errors. Results should be reliable over time (test-retest), between different test leaders (inter-rater) and within the same test leader (intra-rater). Results should also show internal consistency, i.e. across items, such as different machines or different items within a test.

Validity refers to whether an instrument measures what it is set out to measure, without systematic errors. It can be referred to as the accuracy of the results or truthfulness. Validity can be further divided into internal or external validity. Internal validity refers to the interpretation of the results for an individual in the study and whether results can be repeated. Selection of cases, data recording and analysis all affect the internal validity. External validity refers to the generalisability of the results in a population outside of the study.

Imaging methods

The GE lunar iDXA is highly reliable with a correlation coefficient of 0.99 between repeated measurements; this applies to appendicular lean mass and fat mass\textsuperscript{114} as well as full body bone content and density\textsuperscript{115,116}. The same brand
was used at all three sites to guarantee internal consistency. The recommended reference material (NHANES) is from a North American population and it may not be representative for a European cohort. This claim is supported by the fact that the full body BMD T-score in our control group, that ought to have an average of zero, had a mean of 0.9 ± 1.0. Also, in the recent LEAD study that provided reference material for GE Lunar Prodigy DXA, references differed substantially between the two cohorts\textsuperscript{117}. However, a Swedish reference group was provided to increase further the internal validity.

Regarding pQCT, computer tomography derived measures of soft tissue area are quite accurate, and correlate strongly (r = 0.99) with both MR-derived measures of soft tissue area and cadaver measurements at corresponding sites\textsuperscript{97}. SSI predicts approximately 80 – 85 % of the force required to fracture the bone at the diaphysis in a bending test\textsuperscript{95}. The greatest limitation with pQCT is the lack of reference values, which restricts the use to research settings such as these.

**Muscle function**

Peak isometric hand grip strength has a high inter-rater reliability as well as test-retest reliability\textsuperscript{99}. Elbow flexion has also been validated, although in a sitting manner, with high intra-rater and inter-rater reliability\textsuperscript{100}. Also, isometric knee extension has shown a high test-retest reliability\textsuperscript{101}.

Regarding peak isometric strength of the plantar flexors, the weak correlation with muscle CSA in the calf as well as large variation between the three tries leads us to question the reliability of the test. This variance may be partly due to slight flexibilities in the padded plate behind the back, the support straps and the shoe, which could result in the test not being fully isometric. Therefore, test results related to plantar flexion were interpreted with caution and validation is suggested before usage in future studies.

**Cycle ergometer exercise tests**

Data on cycle ergometer exercise tests were extracted from medical journals. The majority of the tests were made at a department for clinical physiology in a highly standardized manner. Also, the overall reduction of exercise
capacity was in line with previous studies. On the other hand, the tests were performed by different examiners that, theoretically, could refrain from pushing the patients to their limits due to their underlying heart condition. Also, the cycle ergometer exercise tests were not done at the precise same time as the imaging and strength testing. Hence, the cardiovascular endurance could have changed between examinations.

**Self-reported physical activity**

There is always a high level of uncertainty regarding self-reported measures, not the least when it comes to physical activity. The IPAQ short form has an acceptable test-retest reliability and a fair internal validity against physical activity measured with accelerometers. We preferred the short form since the long form is more time consuming to fill in and has not proven to be more accurate. However, the IPAQ long form has been studied in CHD, concluding that patients with complex CHD overestimate their physical activity level, although to the same extent as controls.

Numerous questionnaires exist to assess physical activity, such as the IPAQ. To our knowledge, no validated forms exist for assessing resistance training specifically. Hence, in the pQCT study, participants were interviewed about weekly participation in exercise training, both in terms of aerobic exercise training and resistance training separately. It should be acknowledged that this is not a validated method and that the results should not be used more than to generate hypotheses at its best.

In summary, self-reported measures of physical activity should be interpreted with caution. To truly assess the effect of physical activity in general, and physical exercise training in particular, upon body composition, interventional studies would be preferable. Such studies would provide insight into the effect of physical activity on body composition in adults with complex CHD. It would also evaluate the role of physical exercise training in the treatment of pathological deviations of body composition.

**Generalisability**

The external validity ought to be good in countries with health care systems similar to Sweden’s. Since all adults with complex diagnoses of CHD are
followed up within the participating cardiology clinics, irrespective of symptoms or severity of disease, the selection in the studies was not biased towards more symptomatic individuals. This is also supported by the fact that the majority of patients were in NYHA class I. Additionally, we recruited more than half of the eligible patients in the northern region of Sweden, and the patients declining participation did not differ from those included regarding age, sex and diagnosis.

However, variations in phenotype between and within the diagnoses of complex CHD are large. Not just because included lesions differ in severity and hemodynamic consequences, but also due to the evolvement and improvement of surgical methods over time. A better long-term outcome due to a new surgical technique, as well as less reduced exercise capacity, are the reasons d-TGA operated with arterial switch were excluded in the pQCT study. An opposite example is that the subgroup of patients with Fontan circulation potentially could stand for the overall differences between patients and controls. The proportion of patients with a Fontan circulation in the material was 31 %, a larger proportion than in the group of patients with complex CHD in general. Although moderate in size, the materials do not allow for subgroup analyses of all diagnoses of complex CHD. However, the key variables ALM-index and SSI did differ between patients with Fontan circulation and controls, as well as between the group of other diagnoses of complex CHD and controls in the ANOVA, although not to the same extent.

A diagnosis of Tetralogy of Fallot is considered by some moderately complex. Patients with Tetralogy of Fallot consists of four malformations and are cyanotic until corrective surgery. Due to risk of valvular regurgitations, ventricular dysfunction and need for re-interventions, they require follow-up at a ACHD centre. Consequently, they also have a lower exercise capacity and double standardized mortality ratio compared to controls. Not to include patients with Tetralogy of Fallot in studies such as these increases the risk of missing out on important pathologic changes and decreases the quality of care.

In summary, a diagnosis of complex CHD ought to increase the risk for sarcopenia and reduced bone strength on a group level. However, as always,
individualized screening and intervention will be required, as will be discussed further ahead.

**Causality**

The greatest limitation with the cross-sectional design is that it disallows conclusions about causality. Therefore, the only aim for the regression models for muscle quality and bone strength respectively, is to study associations between variables within the patients. Possible causes for the alterations in body composition are discussed, but should be looked upon as hypotheses generating for future longitudinal studies.

**Main results**

**Muscle mass and sarcopenia**

As for our first aim, to describe the elements of sarcopenia in an adult population of complex CHD, we found that more than half of the patients present one or multiple traits of sarcopenia – low muscle mass, low muscular strength and reduced physical performance.

**Muscle mass**

Low muscle mass was confirmed with two different imaging techniques. With DXA, subject to cut-off values, the incidence in the adult patients with CHD was 51 %. This is in line with low leg lean mass with DXA in a previous study of children and adolescents with Fontan physiology. The decreased skeletal muscle mass may also explain why the obesity paradox applies to adults with CHD. The obesity paradox states that a higher BMI has a lower all-cause mortality. The explanation is a low skeletal muscle mass in patients with a normal weight as measured with BMI.

With pQCT, a lower muscle CSA was seen in the forearm in both sexes as well as in the calf in male patients, even after adjusting for height. We propose that the reason female patients did not show lower muscle CSA in the calf was that although they were shorter they did not weigh less. This increased the relative load on their postural calf muscle, which thereby may have affected their calf muscle CSA.
**Muscle strength**

Low muscular strength was confirmed with four out of five different tests. Hand grip strength in the dominant hand, subject to cut-off values, was critically low in 19% of the patients. This is in line with previous studies of low muscular strength in respiratory muscles, knee flexion and hand grip in ACHD\(^{58,122}\).

**Physical performance**

Reduced physical performance, below 75% of expected, was confirmed in 54% of the patients. This is in line with previous studies that report a mean peak \(\dot{V}O_2\) at 43 – 72% of expected in adults with various forms of complex CHD\(^{42}\).

**Lifetime perspective on sarcopenia**

All traits of sarcopenia are associated with a bad prognosis; low muscle mass is associated with decreased survival in a number of conditions such as postsurgery, in patients with renal disease and in patients that undergo cancer treatment\(^{123}\). Low muscle strength in knee flexion predicts death in congestive heart failure and low grip strength is a strong predictor of death in healthy individuals\(^{124,125}\). Decreased physical performance has been associated with increased mortality in patients with CHD\(^{55}\).

Although most frequently described in a geriatric context, sarcopenia associated to CHD is better looked upon from a life-time perspective\(^{126}\). Figure 22 illustrates this, modified from WHO 2003 and Sayer 2008\(^{127,128}\). Since the prevalence of sarcopenia is high in our young cohort of patients with complex CHD, the search for causes should start early in life. Also, in contrast to primary sarcopenia, the mechanisms contributing to sarcopenia in complex CHD affect the individual not just for a longer period of time but also during the important body development in all phases of growth, from infancy to adolescence.
Pathogenetic mechanisms

In my experience, sarcopenic traits are often attributed to low levels of physical activity during youth and adolescence. Indeed, children and adolescents with complex CHD often report a low degree of physical activity as well as reduced confidence in performing exercise training\textsuperscript{129, 130}. However, there are no studies actually measuring the amount of physical activity in patients with complex CHD to support such claims. A Swedish study of children and adolescents with Fontan circulation did indeed report a lower level of self-reported physical activity, but objective measurements showed that they were equally physically active as controls\textsuperscript{131}. Also, in a Canadian study of children with coarctation of the aorta and Tetralogy of Fallot, no differences in any level of physical activity was found compared to controls\textsuperscript{132}. Neither were there any differences in physical activity in a Swedish study of adults with mixed lesions compared to controls\textsuperscript{119}. That said, study populations, especially in studies in children, are small. Also, assessing physical activity in general, and weight bearing exercise in particular, is hard, and differences are likely to be missed. The present studies did not reveal any differences in habitual physical activity between patients and controls, but they did find an
association with ALM-index in simple linear regressions. Also, female patients did indeed report a lower amount of exercise training in general and resistance training in particular compared to the controls. However, the accuracy of self-reported exercise training is scarce as illustrated by the conflicting results of an even negative association between exercise training and ALM-index in male patients, and no associations in either female patients or any corresponding group of controls.

Although low levels of physical activity might not be the primary cause of sarcopenia in complex CHD, a high activity level might slow any further decline. In a longitudinal Icelandic study, sarcopenia developed in a higher degree in participants with the lowest level of physical activity compared to those with a moderate to vigorous physical activity level\textsuperscript{133}.

Of note, although there are sufficient studies proving that aerobic exercise training enhances peak \( \dot{V}O_2 \) in adults with complex CHD, there is only one study in Fontan patients (\( n = 11 \), half of which were randomized to intervention) that have shown that resistance training increases muscle strength and mass in this specific subgroup of complex CHD\textsuperscript{134}. So, whether patients with complex CHD have the same response to muscle training as healthy controls is open for further studies.

Most children with complex CHD are cyanotic, at least until major surgical intervention, which could affect the development of the skeletal muscle\textsuperscript{135}. Numerous studies show that patients with cystic fibrosis, a chronic progressive pulmonary disorder, also have a lower lean mass compared to controls. Also, in cystic fibrosis, low lean mass is associated with decreased pulmonary capacity and indirectly with arterial oxygen saturation\textsuperscript{136}. We found an association between current oxygen saturation and muscle mass in terms of ALM-index in the DXA study. The results were not consistent in multivariable linear regression with habitual physical activity, probably since habitual physical activity is also affected by low arterial oxygen saturation.

Complex CHD is often associated with reduced cardiac output. So is congestive heart failure, a well-known cause of secondary sarcopenia\textsuperscript{137}. Several mechanisms are thought to contribute to sarcopenia in congestive heart failure such as chronic low grade inflammation\textsuperscript{138}, malabsorption due to gastrointestinal oedema\textsuperscript{139}, and the limited cardiac output during exercise\textsuperscript{140}. The
same mechanisms ought to contribute to sarcopenia in complex CHD, just for a longer period of time and during the bodily development.

As to further explore causation to low muscle mass, longitudinal studies are gold standard. However, skeletal muscle biopsies and histology could also be valuable in distinguishing between untrained and pathologic histology.

**How to assess sarcopenia in clinical practice**

A simple way of assessing sarcopenia in clinical practice could be according to the European Working Group on Sarcopenia in Older People (EWGSOP) algorithm modified to an ACHD context as in figure 23⁶⁹: sarcopenia should be suspected in all patients with complex CHD and a screening for decreased muscular function with grip strength would diagnose probable sarcopenia.

Grip strength is the best validated as well as the most easily assessable of the tests described; hence, it should be preferred. Also, grip strength correlates strongly with exercise capacity and could possibly be used instead of the latter. Finally, mounting evidence proves that grip strength alone is a good prognostic marker not only of cardiovascular disease but of all cause death in diverse populations¹¹¹,¹⁴¹.

In clinical practice, low grip strength could be enough to start an intervention such as optimizing the patient with regard to diagnosis, ensure proper nutritional intake and prescribing individualized resistance exercise training¹⁴². Sarcopenia could then be further confirmed with DXA and the severity determined by exercise capacity⁶⁹.

Alternatively, exercise capacity, already assessed in clinical practice using CPET or cycle ergometer tests, could be used along with grip strength to identify patients at risk of a worse prognosis. When analysing sarcopenic traits in the overlap between the two studies (figure 21), all patients with low grip-strength also had a decreased exercise capacity, but not the other way around. This indicates that grip strength might be highly specific but not sensitive enough in identifying sarcopenia in adults with complex CHD and is best used in combination with exercise capacity.

Note that these are merely our suggestions of how sarcopenia could be dealt with in clinical practice, when further studies have evaluated both predictive
values of the methods alone and in combination as well as the effect of resistance training.

![Diagram of Algorithm](image)

**Figure 23 Suggested algorithm for assessing sarcopenia in clinical practice, modified after the European Working Group on Sarcopenia in Older People (EWGSOP) algorithm.**

**Bone health**

As for the second aim to describe, within an adult population with complex CHD, the elements of bone quality: reduced bone mass, bone density and bone strength were found. There was no increase of the traditional risk factors for osteoporosis, except the use of loop diuretics and warfarin compared to the control group. The only factor associated with reduced bone quality was shorter height.

**BMC and BMD**

Full body bone mass was found to be lower using DXA, as well as the radii and tibiae bone mass using pQCT. Full body bone density was equally found
to be reduced using DXA, (despite adjusting for the patients’ slightly shorter stature with linear regression), but was not in the radius and tibia using pQCT. The reason for the discrepancy could be either a limitation in the linear model or that the two methods measure at different sites, full body vs. regionally in the limbs, and bone density can vary between these.

SSI

Regardless of bone density, the patients showed a reduced mechanical strength in the tibia in terms of SSI compared to controls. The structural parameters causing the differences were that patients’ tibiae cortex was thinner, due to a smaller outer bone diameter. Since the outer perimeter of the bone is established during pubertal growth, a smaller such could indicate disuse or disease at an early age\textsuperscript{143, 144}. The trait, with normal cortical BMD and reduced SSI with pQCT in equally tall peers, is also seen in other groups of patients with chronic diseases since childhood, such as juvenile arthritis\textsuperscript{145}.

Pathogenetic mechanisms

The shorter stature and reduced outer cortical perimeter of the tibia suggest an impact on bone growth and formation from an early age. Hence, bone health in adults with complex CHD should be looked upon from a lifetime perspective; with prolonged exposure affecting bone formation and limiting peak bone mass as well as possibly a faster decline during further ageing. This is a shared trait with sarcopenia, as is most plausible mechanisms such as cyanosis, renal impairment and heart failure.

Cyanosis is prevalent in complex CHD and is thought to have a direct effect on bone formation\textsuperscript{146}. In the DXA study, there was an association between arterial oxygen saturation and BMDa in the simple linear regression, but not after adjusting for height in the multiple linear regression. This of course could be due to cyanosis affecting skeletal growth, and hence, both height and BMDa. In the pQCT study, arterial oxygen saturation was not associated with SSI. However, the patients in the present studies had a close to normal arterial oxygen saturation, which does not reflect the cyanotic period prior to surgical intervention.

Heart failure is also prevalent in complex CHD\textsuperscript{147} and is associated with decreased bone health in the population in general\textsuperscript{148}. Heart failure, assessed
by systemic ventricular function was not associated with BMDa or tibiae SSI. Like cyanosis, current systemic ventricular function, is not representative of a lifetime of reduced cardiac output. Longitudinal studies are needed to establish not only association but also causation, both between arterial oxygen saturation and reduced bone quality, and between heart failure and bone quality.

Low oxygen saturation as well as heart failure might also affect bone growth through cyanotic nephropathy and renal impairment\textsuperscript{149}. Reduced kidney function leads to a decreased calcium resorption, vitamin D-deficiency and secondary hyperparathyroidism, all previously described in both children and adults with CHD\textsuperscript{67,150,151}. The lack of association between creatinine based eGFR and SSI in the tibia could be due to patients largely having a reduced skeletal muscle mass, a reduced creatinine and thereby a falsely high eGFR.

The patients with complex CHD had a similar amount of traditional risk factors associated with fractures as controls (table 10). However, an increased usage of warfarin and loop diuretics was found. Warfarin inhibits vitamin K and could theoretically affect protein synthesis and bone formation, but previous studies on warfarin usage as a risk-factor for fractures are conflicting\textsuperscript{152,153}. Loop diuretics cause calcium loss in the kidneys and affect bone turnover and seem to be associated with increased fracture risk, especially in men\textsuperscript{154}. Loop diuretics have also been studied in the context of children with CHD and heart failure in an intensive care unit, and here it was associated with an increased risk of fractures\textsuperscript{155}. In recent studies, ongoing use of either warfarin or loop diuretics were not associated with decreased bone strength parameters. Since loop diuretics are often used in case of kidney dysfunction and heart failure, both comorbidities associated with fracture risk\textsuperscript{148}, there is always a risk of confounding. Also, one underlying mechanism between the use of loop diuretic and fractures could be decreased blood pressure and hence an increased risk of falling, which we do not assess with either DXA or pQCT.

The only factor, independently associated with lower bone strength was height. Although height could be considered as a confounder to decreased bone strength variables, maybe it should be looked upon as a non-modifiable risk factor for fractures instead. Normally, shorter individuals are at a decreased risk of fractures, especially hip fractures, but then it is most likely
due to a genetic predisposition for shorter height and not a congenital disease that could affect growth and bone development\textsuperscript{156}.

**Bone health and osteoporosis**

Contrary to sarcopenia, the definition of osteoporosis has remained virtually unchanged since first described by WHO in 1994\textsuperscript{109}. Osteoporosis is defined as an BMD\textsubscript{a} from the femoral neck of 2.5 SD or more below the young female adult mean, using normative data from women aged 20–29 years\textsuperscript{157}. Hence, neither the BMD\textsubscript{a} from full body DXA, nor strength parameters from pQCT, as in the present studies, can be used to claim a higher prevalence of osteoporosis in adult patients with complex CHD.

What can be claimed though is an impaired bone quality, possibly increasing the risk of future fractures with further decline. I have only found one study on fracture incidence in children and young adults with CHD (mean age 26.6 years). In this register-based study, in the subgroup of patients with complex lesions, the incidence of fractures was actually lower than in the reference group\textsuperscript{158}. The authors speculate that patients were restricted in terms of physical exercise, and hence reduced their risk of fractures in the short term but increased it in the long term\textsuperscript{158}. Most likely, the studied population was far too young to draw any conclusions about fragility fractures, since these ought to be quite rare for this age group.

**Clinicians’ perspective on bone health**

The two most important risk factors for fragility fractures are age and previous fragility fractures\textsuperscript{159}. However, it seems unwise to wait for these to happen. In expectation of future studies, cardiologists handling the “young” adult patients with complex CHD should pay close attention to modifiable risk factors for osteoporosis and fractures. In addition to optimizing heart failure and renal disease, this includes reducing risk factors such as smoking, alcohol use and use of glucocorticoids\textsuperscript{160}. The online “Fracture Risk Assessment Tool” (FRAX) provides country-specific algorithms for estimating individualized 10-year probability of hip and major osteoporotic fracture, and can be very useful\textsuperscript{161}. FRAX identifies risk factors and patients benefitting from further evaluation with DXA and bone specific treatment\textsuperscript{160}.
Calcium and Vitamin-D should be supplemented if decreased, or in case of manifest osteoporosis; otherwise not.

Physical exercise should be encouraged throughout life. Although it is never too late to start exercising, it should be emphasised that the bone strengthening effect of exercise training is greater in childhood and adolescence than later in life. It is also evident that the best bone strengthening effect comes from high-intensity resistance exercise and impact training, at least in older individuals. However, physical exercise and activity do not just have a bone strengthening effect, but also increase strength and enhance coordination and thereby decreases the risk of falling.

**Fat and sex differences**

In this adult population of complex CHD, female patients had an increased amount of fat mass as well as increased proportion of fat mass compared to controls. The fat mass was not located in the limbs, nor were there any signs of excess intramuscular fat in terms of muscle density. Instead, the excess fat was predominantly distributed around the internal organs. This was illustrated by high amounts of VAT and fat mass in the android regions in the DXA study as well as a high waist/hip-ratio in the pQCT study. Compared to subcutaneous fat, VAT secretes free fatty acids to a higher degree, with direct access to the liver through the portal vein. A high amount of VAT is associated with an increased risk of metabolic disease such as diabetes and cardiovascular disease. Moreover, the higher fat mass occurred without a proportional increase in muscle mass, which is reflected in the high prevalence of sarcopenia, i.e. sarcopenic obesity. Do note that the increase in fat mass was not reflected in an increased prevalence of overweight or obesity, as defined by BMI, and could therefore be missed.

Sex differences in outcomes in CHD are described previously. For instance, women with CHD have an increased prevalence of pulmonary hypertension and a lower risk of receiving an implantable cardiac defibrillator. But these results, with an increase in fat mass as well as an unhealthy distribution thereof in women with complex CHD, need confirmation in other studies.

The female patients did indeed report weekly participation in exercise training and resistance training in respective study to a lesser extent than the control
groups. Hence, as always, the differences could be due to a more sedentary lifestyle.

Other explanations ought to be sought in hormonal changes or gender differences. The latter is related to social constructs causing society to treat girls and boys as well as women and men differently. One example could be that parents and health personnel are more overprotective towards baby girls with CHD than boys in terms of physical activity.

To my slight surprise, many studies in CHD still do not even take sex into consideration when exploring anthropometric differences\textsuperscript{44}. Moreover, I have found no qualitative studies exploring gender differences in CHD; apparently an open field of research.

**Further research**

Researchers always suggest numerous future research projects. This thesis is no exception. However, if I had to emphasize a few, providing clinically important knowledge, it would be the following:

- Screen all adult patients with complex CHD for sarcopenia using grip strength and follow-up within existing quality registers such as SWEDCON. Evaluate grip strength as a prognostic tool for morbidity and mortality along with exercise testing. Do note the importance of adjusting for height.
- Evaluate the effect of resistance training in adults with complex CHD with regard to muscular strength and muscle mass. Compare with a healthy control group.
- Adult patients with complex CHD have decreased bone health and complex CHD might be a risk factor for osteoporosis and future fractures. Future studies, as patients grow older, should verify if this is the case. In studies on bone health, include biomarkers such as calcium and vitamin-D.
- Increased fat mass in female patients with complex CHD calls for confirmation in further studies. If confirmed, the different deviations in body composition could be due to gender – an open field of research in CHD.
Conclusion

Adults with complex CHD have:

1) Reduced muscle mass, muscular strength and physical performance and a high prevalence of sarcopenia (papers I and III).

2) Reduced bone mass and bone mineral density assessed with DXA and reduced bone strength in the legs assessed with pQCT (papers II and IV). The only traditional risk factors for osteoporosis over-represented in patients was the usage of loop diuretics and warfarin, none of which was associated with lower bone strength (papers II and IV).

3) A high proportion of fat mass in women, predominantly located around internal organs in terms of visceral adipose tissue (papers I and III).
A final reflection

Complex CHD has long reaching effects beyond the heart. Hence, the cardiologist has to look beyond the heart as well. At the least, if we shall continue to write the story of success of the survivors with complex CHD.

Still, when engaging in conversation with participating patients, some declined information about the results. My impression, although not systematically evaluated within the frames of the study, was that they already felt sick enough without having it thrown “in their face” or that they “did not want to know everything that might go wrong in the future”. This, of course, was respected without further ado.

Some patients, on the other hand, were well aware of their prognosis and were eager in taking part in both their individual performance as well as study results, hoping to optimize their lifestyle and risk factors.

This calls for consideration when trying to engage patients in preventive measures, both pharmaceutical and life-style-related; perhaps best expressed in the famous words of Danish philosopher Søren Kierkegaard:

“If one is truly to succeed in leading a person to a specific place,
one must first and foremost take care to find her where she is and begin there”
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