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FACULTY OF MEDICINE AND LIFE SCIENCES

Master of Biomedical Sciences

Master's thesis

Recovery in cardiopulmonary and oxidative muscular function during exercise after minimally invasive aortic valve replacement

Supervisor :
Prof. dr. Dominique HANSEN

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Dr. ALAADDIN YILMAZ

Hajar Boujemaa

Thesis presented in fulfillment of the requirements for the degree of Master of Biomedical Sciences

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Hajar Boujemaa
08/06/2017
Ham

List of abbreviations

ACC/AHA: American Colleges of Cardiology/American Heart Association
AVR: Aortic valve replacement
AVA: Aortic valve area
AS: Aortic stenosis
A mitral: Trans-mitral peak early diastolic velocity
BP: Blood pressure
CO: Cardiac output
COi: Cardiac output index
CPET: Cardiopulmonary exercise test
CVD: Cardiovascular disease
E mitral: Trans-mitral peak early diastolic velocity
E': Early diastolic mitral annular velocity
ECG: Electrocardiogram
EF: Ejection fraction
EqCO2: Equivalent of CO2
EqO2: Equivalent of O2
HR: Heart rate
LV: Left ventricle
LA: Left atrium
Max-CPET: Maximal cardiopulmonary exercise test
Mini-AVR: Minimally invasive aortic valve replacement
MRT: Mean response time
O2: Oxygen
O2-pulse: Oxygen pulse
PAPs: Pulmonary hypertension
PLAX: Parasternal long axis
RPE: Ratings of perceived exertion
RER: Respiratory exchange ratio
SIRs: Systemic inflammatory response syndrome
SM-CPET: Submaximal cardiopulmonary exercise test
TAPSE: Tricuspid annular plane systolic excursion
TEE: Transesophageal echocardiography
TTE: Transthoracic echocardiography
VE: Minute ventilation
VCO2: Carbon dioxide production
VO2: Oxygen uptake
VO2 max: Maximal oxygen uptake
VO2/HR: Oxygen pulse
VT: Tidal volume
W: Cyclin power output

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Abstract

Background:
Aortic valve stenosis (AS) is one of the greatest burdens in elderly people. The only effective treatment is aortic valve replacement by surgery. However, it remains unidentified how the recovery in cardiopulmonary and muscular function after minimally invasive aortic valve replacement (mini-AVR) surgery is manifested. Therefore, the main hypothesis was that "**early after mini-AVR surgery, an improvement in cardiopulmonary function and not in oxidative muscular function is observed**".

Methods:
This was a combination of a cross-sectional and prospective observational study. 14 AS patients were followed for up to 3 weeks after mini-AVR surgery and examined at following time points: pre-operative, 5 days and 3 weeks after mini-AVR surgery were submaximal cardiopulmonary exercise test at 25% of maximum workload, echocardiography and cardiovascular disease risk were assessed. Furthermore, peri-operative parameters were assessed during mini-AVR surgery.

Results:
During exercise, significantly elevated EqO2 and EqCO2 were observed ($p = 0,002$ resp. $p = 0,008$) compared to healthy controls before mini-AVR surgery. Exercise MRT did not differ between these two groups. At five days after surgery both EqO2 and EqCO2 during exercise were significantly elevated ($p = 0,007$ resp. $p = 0,008$). Exercise MRT was significantly slower at five days ($p = 0,036$) versus exercise MRT measured prior to surgery. Three weeks after surgery, EqCO2 during exercise was significantly lower ($p = 0,021$) and normalized compared to pre-operative levels. Accelerated exercise MRT was observed at three weeks in AS patients. Furthermore, cross-clamp time was independently related to exercise MRT at three weeks after mini-AVR surgery ($p = 0,001$).

Conclusion
Systematic significant deterioration in exercise performance was observed after minimally invasive aortic valve replacement, which is probably related to ventilation perfusion mismatch. Need for improved early post-operative treatment is of great importance to prevent worsening of physical condition of these patients.

Table contents

1. Introduction

1.1 Valvular heart diseases: aortic stenosis

Valvular heart diseases are of growing attention in the field of cardiovascular diseases, more in particular aortic valve disease caused by aortic stenosis (AS) (1). In the Western society, aortic valve calcification is the second most common cardiovascular disease after coronary artery disease. In general, it effects the elderly population with a prevalence of 2-10%, increasing strongly with age. It is expected that due to the aging Western population, the number of AS patients will increase dramatically in the future. It is an important cause of high morbidity and mortality rate if left untreated (2). AS, narrowing of the aortic valve, is characterized by slow progressive inflammation and calcification of the heart leaflets (**Figure 1A**). The most frequent cause of AS is buildup of calcium on the aortic valve, but congenital heart defects at birth and rheumatic fever in childhood are also causes of AS (3). At the onset of AS, thickening of the valve leaflets and mild calcification initially takes place which does not cause any symptoms. Over time, thickening and calcification of aortic leaflets becomes more severe, due to chronic inflammation, leading to obstruction of the blood flow (4). This results in remodeling of the left ventricle due to an increase in left ventricular pressure load which has several implications such as left ventricular hypertrophy, left ventricular diastolic and/or systolic dysfunction, congestive heart failure, angina, arrhythmias and syncope (5). Therefore, one of the hallmarks of AS is an impaired cardiac output leading to left ventricular dysfunction and ventilation-perfusion mismatch. In these patients, ventilation is marked by a disproportionate increase to the metabolic needs to compensate for inadequate perfusion (6). As a consequence, patients with AS have an impaired hemodynamic response to exercise (7).

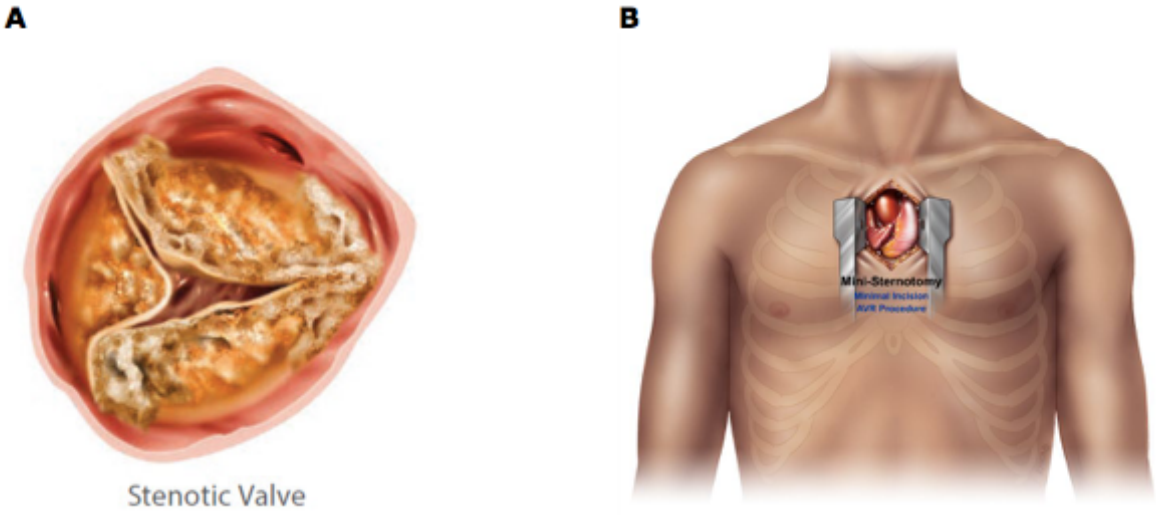

Figure 1: Valvular heart disease and minimal invasive surgery
A: Severe calcified aortic valve ; B: minimal invasive aortic valve (mini-AVR) surgery through hemi-sternotomy

Most patients remain asymptomatic, but as soon as symptoms like dyspnea, angina or dizziness occur, the patients' outcome worsens over a short period of time (2-3 years) (8-10). Criteria of

severity of AS are determined by the American Colleges of Cardiology/American Heart Association (ACC/AHA) using aortic valve area (AVA) as a parameter. AVA is assessed by echocardiography. Echocardiography has evolved as a non-invasive key tool for diagnosis and evaluation to assess valve stenosis (11). An AVA >1,5 cm^2 is considered as mild AS, an AVA between 1,0 cm^2 and 1,5 cm^2 is considered as moderate AS and AVA <1,0 cm^2 is considered as severe AS (12).
Not only the heart function is affected in patients with severe AS due to left ventricular dysfunction. There is also an impact on the ventilatory function of these patients. Severe AS leads to an increased blood retention in the left side of the heart resulting in pulmonary congestion. This results in pulmonary hypertension leading to a decreased maximal oxygen uptake which is manifested in breathlessness (13). On the other hand, severe AS has also an impact on the peripheral muscular function. Previous studies already demonstrated that there is an alteration of the skeletal muscle mitochondrial function. This can be due to reduced physical activity and sedentary behavior, skeletal muscle fatigue and muscle weakness which is associated with early anaerobic metabolism in the skeletal muscle (14).

Because there is no pharmacological treatment available to treat or prevent further development of AS, the only effective treatment of severe AS is surgery. Replacement of the aortic valve is highly effective in improving morbidity, mortality as well as the patients' outcome (15). Over the last decade, minimally invasive aortic valve replacement (mini-AVR), has been used more often to replace the calcified aortic valve via hemi sternotomy instead of conventional surgery where full sternotomy is performed (**Figure 1B**) (16). The aim of this surgical approach is to maintain the efficacy, safety and quality of a conventional approach, but in a less invasive way (17). Although total cardiopulmonary bypass is still needed when performing mini-AVR, this new surgical approach leads to many advantages, improved post-operative outcomes and improved quality of life of the patient. These advantages include reduced pain, reduced surgical trauma, faster recovery, improved cosmetics, decreased risk of infection, reduced incidence of arrhythmias and reduced costs (18-20). Despite that mini-AVR has various benefits and has been performed increasingly over the last decade, scientific assessments of this surgical approach on the outcome of the patient after this surgery have been lacking.

Nowadays, when the outcome of minimally invasive surgical approaches is evaluated, the focus is mostly on 'hard endpoints' (morbidity and mortality) only, while functional outcome measures ('softer endpoints') are rarely considered. The consideration of these 'softer endpoints', after mini-AVR, can be of great importance to make intervention possible in cases of abnormal recovery, even before the appearance of symptoms. This could play a key role in the follow-up of elderly patients who receive a new aortic valve. It is known from previous studies that elderly patients have a slow and often complicated recovery after surgery (21, 22). This can be explained by the fact that aging is associated with a loss of function in many systems of the body. Because of surgical stress, a systemic inflammatory response can occur which is related with worse clinical outcome and presence of several comorbidities. For this reason, these patients are more prone to develop surgical and medical complications in the weeks and months after surgery (23-25). Therefore, these 'softer endpoints' can have a prognostic value and can give insight in how post-operative recovery of AS patients is manifested (26). Today, there is a lack of information that gives some insight in how the

recovery is manifested in patients who had mini-AVR surgery. In particular, the knowledge about changes in cardiac, pulmonary and oxidative muscular function after mini-AVR is lacking. Strikingly, this information has never even been assessed in conventional surgery. Therefore, additional research is required to identify the impact of mini-AVR surgery on certain changes on cardiopulmonary and oxidative muscular function.

1.2 Exercise testing

Changes during recovery in cardiac, pulmonary and oxidative muscular function after mini-AVR can be assessed by executing exercise testing. Previous studies have already shown that exercise testing can provide valuable information predicting worse outcomes of patients that are in need of a new aortic valve (27, 28). Cardiopulmonary exercise testing (CPET), in this attempt, has emerged as an important functional assessment tool that has a diagnostic (symptomatic state) as well as a prognostic (risk of events) value and is able to provide risk assessment (29). CPET is a non-invasive method that measures a broad range of variables associated with cardiopulmonary and muscular functions in patients with cardiovascular and pulmonary disease. The primary purpose of CPET is to gain insight into quantitative cardiac, pulmonary and metabolic responses to exercise. These responses can be obtained through breath-by-breath analysis and 12 lead electrocardiogram (ECG) (6, 30). CPET also provides breath-by-breath gas exchange measures of three variables: oxygen uptake (VO2), carbon dioxide output (VCO2) and ventilation (VE) which are used to determine other gas exchange patterns reflecting the response of the patient on exercise (31). Cardiac function is analyzed by assessing heart rate (HR), 12 lead ECG with ST analysis, blood pressure and oxygen pulse (VO2/HR; O2 pulse), a good stimulator of cardiac stroke volume. Ventilatory function is analyzed by the assessment of minute ventilation (VE), tidal volume (VT), equivalent of O2 (VE/VO2), equivalent of CO2 (VE/VCO2) and respiratory rate (32). Thus, CPET is a combination of exercise testing procedures (e.g. ECG, blood pressure) along with ventilatory expired gas analysis (VO2, VCO2 and VE). Therefore, CPET is highly valuable to isolate physiologic abnormalities in the cardiac, pulmonary and oxidative muscular function.

In clinical settings, maximal CPET (max-CPET) tests are often conducted to evaluate exercise capacity and predict outcome of patients with heart failure and other cardiac conditions. During peak exercise, measurement of VO2 is used to assess functional capacity of the patient. Peak exercise capacity is characterized as "the maximum ability of the cardiovascular system to deliver oxygen to exercising skeletal muscle and of exercising muscle to extract oxygen from the blood" (33). Peak VO2 value is therefore an important predictor of prognosis in patients with heart failure and many other patient populations (31). Although max-CPET is often considered as the gold standard for assessing maximal aerobic capacity of the patient, it may not be suited for patients who suffer from severe AS. Patients with AS are not allowed to execute a maximal exercise test due to higher risks for acute adverse events. Submaximal CPET (SM-CPET) is for that reason more adequate and is of growing interest as it reflects the functional capacity of these patients very well. Additionally, SM-CPET measures in elderly patients with AS, in particular assessment of VO2 kinetics, can predict clinical cardiac outcomes and monitor changes in functional capacity (34, 35).

1.3 Oxygen uptake kinetics

The rate of change in VO2 before, during and after exercise can be described best by VO2 kinetics when SM-CPET is performed. Both cardiac output and systemic O2 extractions (arterial 02 content and O2 utilization) determine VO2. For that reason, VO2 onset and recovery kinetics can be used to determine the cardiac, pulmonary and muscular functions during and after submaximal exercise (36). VO2 kinetics in SM-CPET have been shown to be valuable for objective evaluation of functional capacity of several patient populations (e.g. multiple-sclerosis patients, heart failure, …). VO2 kinetics define the change of O2 uptake during onset and recovery of SM-CPET at a constant load (37).

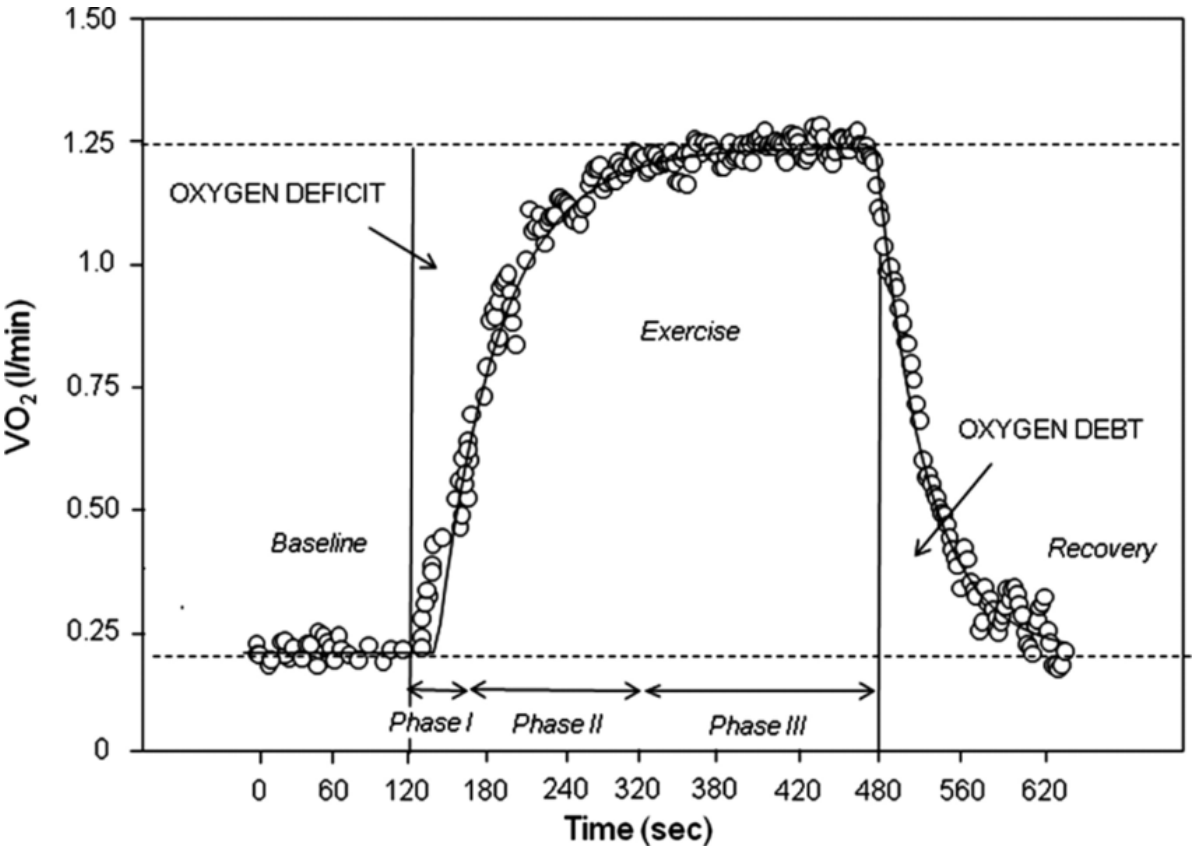

Figure 2: Oxygen uptake (VO2) kinetics in submaximal cardiopulmonary exercise test (SM-CPET) (6). VO2 Kinetics as a function of time during constant work load in a healthy subject helps to define the physiological background leading to 02 uptake. It can be divided in to phase I (cardiodynamic), phase II (cell respiration) and phase III (steady state).

1.3.1 Phase I: Cardiodynamic phase

During SM-CPET with constant load, VO2 kinetics are characterized by three phases (**Figure 2**). In phase I, cardiodynamic phase, a fast VO2 kinetic increases is normally observed during exercise when a constant workload is applied. This increase is related to the increase of pulmonary blood flow caused by a higher cardiac output. A case study showed that this first phase of VO2 kinetic is altered in patients who have severe AS because the increase of VO2 kinetic is significantly decreased (38)

(**Figure 3**). This decrease reflects the inability in patients with severe AS to increase their cardiac output as a result of left ventricular dysfunction. This leads to a limitation of the cardiopulmonary reserve for exercise in patients with severe AS. This limitation does not only has an impact on exercise tolerance but affects also the recovery after exercise (3).

1.3.2 Phase II: Cell respiration phase

Phase II, cell respiration, demonstrates a mono-exponential increase in VO2 and is characterized by a rapid exponential increase of VO2 (**Figure 2**). The initiation of this phase is the result when venous blood from muscles arrives at the lungs. For that reason, pulmonary VO2 reflect the muscle VO2 kinetics (36, 39). Patients who have a reduced exercise capacity, can have possible skeletal muscle adaptations. It has been shown that a decreased exercise tolerance in heart failure patients is related to skeletal muscle abnormalities that could induce early anaerobic metabolism (40). Oxidative enzyme change in active tissue is measured by VO2 at the mouth and therefore the VO2 response rate at the onset of exercise reflects muscle metabolism and systemic oxygen transport (41). A significant correlation between exercise onset VO2 kinetics and max VO2 was found by Powers *et al.* (42). Moreover, exercise-onset VO2 kinetics are known to be faster in slow-twitch skeletal muscle fibers and with increased enzymes change, activation of oxidative muscle is accelerated (43, 44).

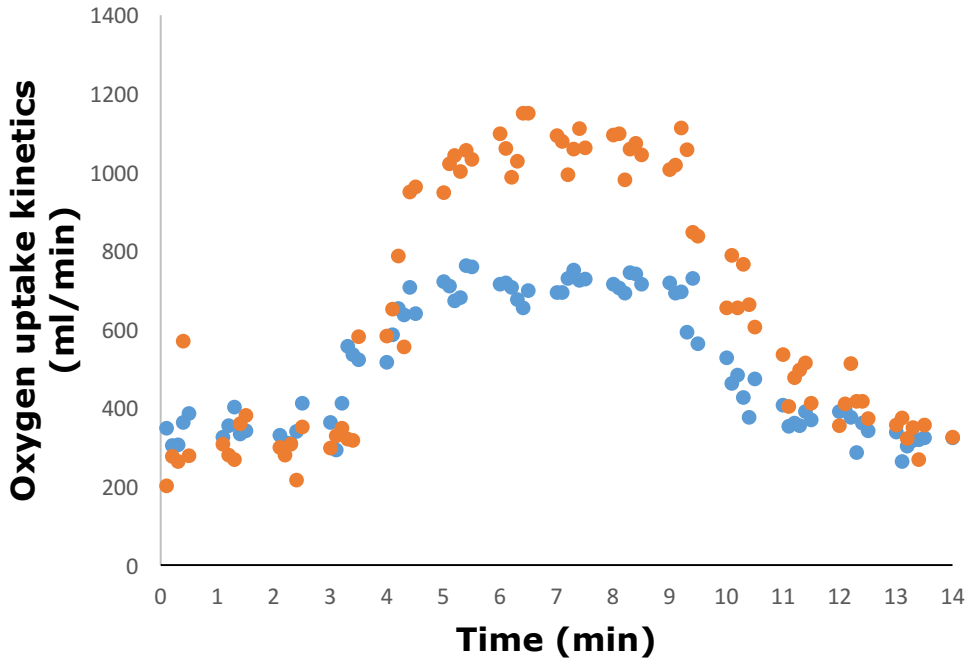

Figure 3: Oxygen uptake (VO2) kinetics in healthy subject and aortic stenosis (AS) patient.
Own data of VO2 course during and after a constant work load at submaximal intensity (25% Wmax) in a healthy subject (orange) and in a patient with severe AS (blue) is shown. A six min exercise bout started at three minutes followed by a recovery time of six minutes.

1.3.3 Phase III: Steady-state

Phase III, final phase of VO2 kinetics, is characterized by a steady-state and is conform to the point at which VO2 and cardiac output reach a plateau (**Figure 2**). At submaximal intensity with a constant

workload, steady-state in VO2 is normally reached within the first 3 min (36, 37). The increase to steady-state of VO2 kinetics are known to be significantly slower in patients with vascular diseases (**Figure 3**) (38, 45). In exercise with constant workload, O2 requirements are assumed to be the same as the steady state of VO2 (46).

Exercise VO2 kinetics is a sensitive tool for the evaluation of oxidative muscle function and functional capacity of AS patients (47). Therefore, it is of great importance to gain more knowledge about the physiological determinants of VO2 kinetics. This may lead to the understanding of several functional impairments caused by pathophysiological mechanisms in AS patients and after mini-AVR surgery. Moreover, this could lead to implementation of therapeutic approaches that lead to an improved exercise capacity in patients who underwent mini-AVR surgery.

In conclusion, the evaluation of the functional capacity in severe AS patients is not routinely executed in clinical practice after mini-AVR surgery. The absence of standardization of the methodology and a lack of studies which evaluate the clinical use of VO2 kinetics in AS patients are possible reasons which can explain why this is not routinely included in clinical practice. Moreover, the etiology of pathophysiological mechanisms underlying the change in VO2 kinetics in AS patients is not understood. Therefore, assessment of changes in cardiopulmonary and oxidative muscular function can provide insights in post-operative recovery after mini-AVR surgery. This leads to the understanding in the need for additional interventions that have to be implemented to improve post-operative recovery. Moreover, pre-operative CPET can be used to predict peri- and post-operative outcomes. In previous studies, changes in physical fitness in patients who underwent mini-AVR surgery have been assessed during rehabilitation (48, 49). To our knowledge, studies assessing changes during exercise in cardiopulmonary and oxidative muscular function after aortic valve replacement (AVR) are absent.

1.4 Hypothesis and objectives

The aim of this study was to investigate the effect of mini-AVR surgery on the early changes in cardiopulmonary and oxidative muscular function in AS patients and to gain insight in parameters that may predict a worse outcome within the first three weeks after surgery.

It is hypothesized that cardiopulmonary function after mini-AVR surgery is significantly improved but not oxidative muscular function, and that a worse prognosis can be predicted if abnormal recovery of cardiopulmonary function occurred within the first three weeks. To investigate this hypothesis, following objectives are assessed:

1. First, changes in cardiopulmonary and oxidative muscular function in early stage (within first three weeks) during exercise after mini-AVR surgery are determined.

2. Subsequently, prognostic potential of early changes (within first three weeks) in cardiopulmonary and oxidative muscular function during exercise after mini-AVR are described.

3. Pre-, peri- and post-operative factors related to anomalous recovery in cardiopulmonary and oxidative muscular function during exercise after mini-AVR are explored.

2. Materials and methods

2.1 Ethical approval

The institutional ethical board at Jessa Hospital (Hasselt, Belgium) and Hasselt University (Hasselt, Belgium) approved the research protocol of this study. Signed informed consents of all AS patients and healthy individuals were obtained after explaining the aim of this study.

2.2 Subject selection

This was the first study to our knowledge that has evaluated detailed changes in cardiopulmonary- and oxidative muscular function during exercise after minimal invasive aortic valve replacement (mini-AVR) surgery. Therefore, it was not possible to execute a priori sample calculation. From August 2016 to May 2017, 32 healthy controls and between November 2016 and May 2017, a total of 20 patients were asked to participate to this study. All 32 healthy controls and 14 patients, who were in need of a new aortic valve, agreed to participate and were included in this study (Figure 4). These patients were fully gender-, age- and weight-matched with 14 healthy individuals. 14 patients underwent a SM-CPET the day before mini-AVR surgery. Five days after mini-AVR surgery, a drop-out of five patients was observed (n=9). Three of these patients were unable to complete the first bout of a submaximal exercise test due to ratings of perceived exertion and 2 patients suffered from post-operative complications that resulted in the inability to perform any physical activities. Three weeks after surgery, the same number of drop-out of five patients was observed (n=9). Two patients who were unable to perform an exercise test earlier at five days after surgery were also unable to perform the exercise test at three weeks. The other three patients were able to complete the exercise test at day five after surgery, but at week three rehospitalization took place due to complications (**Figure 4**).

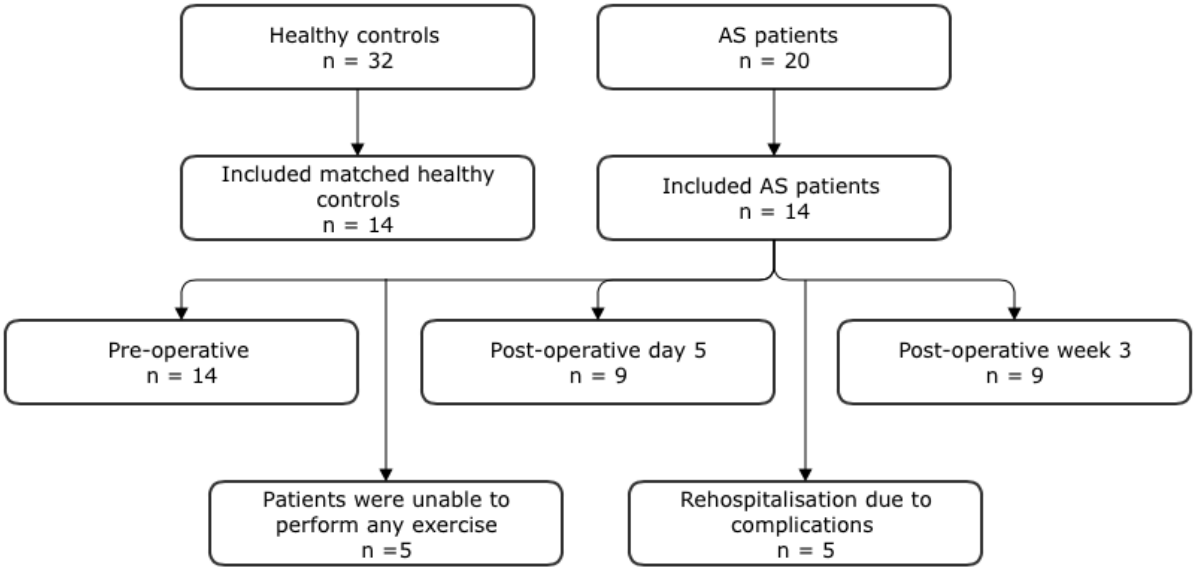

Figure 4: Flowchart of healthy controls and AS patient population.
AS: aortic stenosis

Exclusion criteria for healthy controls included no chronic disease (type I and II diabetes, chronic pulmonary diseases e.g. asthma, chronic obstructive pulmonary disease (COPD), etc.) or exercise limiting conditions (not able to perform an exercise test due to physical limitation). Exclusion criteria for AS patient participation to this study included: no ability to execute an exercise test due to physical limitations, surgery for coronary artery or peripheral disease and no ability to follow a standardized rehabilitation program.

2.3 Study design

This was a combination of a cross-sectional and prospective observational study. Patients were only allowed to participate after the approval of treating cardiologist was obtained. The cross-sectional study consisted of data obtained from SM-CPET, echocardiography, past physical activity and CVD risk factors from AS patients which were compared with age, gender and BMI-matched healthy controls.

The prospective observational study design with corresponding time points and measurements are mentioned in Figure 1. 14 patients were followed for up to 3 weeks after mini-AVR surgery and examined at following time points: pre-operative, 5 days and 3 weeks after surgery. After one month, a 3-month exercise based intervention was started.

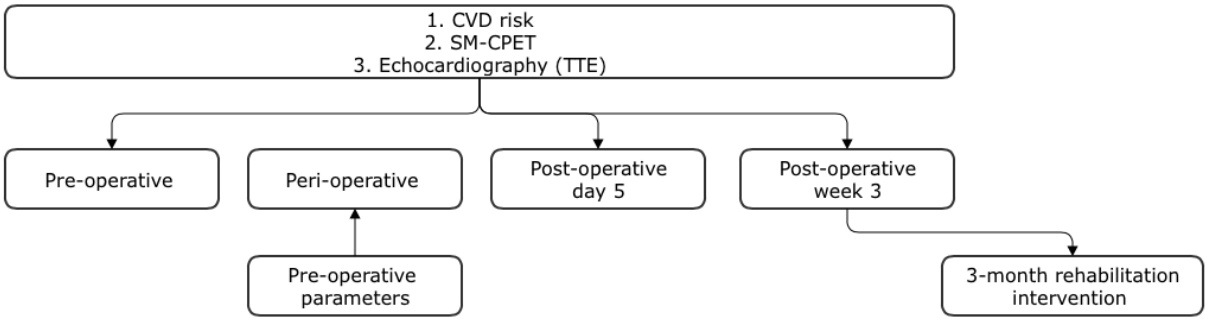

Figure 5: Prospective observational study design
CVD: cardiovascular disease risk; SM CPET: submaximal cardiopulmonary exercise test; TTE: Transthoracic echocardiography

Peri-operative course was standardized: similar anesthesia was applied, extubation of subjects occurred within 4-8 hours after surgery and patients stayed for maximally two days at the ICU and maximally for eight days in regular room. All patients received a similar in-hospital rehabilitation program.

2.4 Cardiovascular disease (CVD) risk

CVD risk was determined by measuring blood pressure (Omron, HEM-7131-E, Omron healthcare Europe B.V., Netherlands) that was measured after subject was laid down for a period of five minutes for three times. The average of these three blood pressure measurements was taken. Next height, body weight (from which BMI is calculated, measured with clothes) and waist circumference between last chest ribs and ilea (automatically spring measuring tape) in healthy individuals and AS patients was measured at following time points: pre-operative, five days and three weeks after surgery.

2.5 Submaximal cardiopulmonary exercise test (SM-CPET)

SM-CPET was performed to obtain detailed information about changes of cardiac, pulmonary and muscular function during exercise. Patients performed a SM-CPET on an electronically braked cycle ergometer (eBike Basic, General Electric GmbH, Bitz, Germany) and were advised not to perform any exercise the day before or the day of testing and only eat a light meal at least two hours prior to testing.

Resting data were obtained when patients were seated on a bike for three minutes and were instructed to cycle between a rate of 60-70 rpm, against a resistance corresponding to 25% of predicted cycling power output (Wmax), for six minutes. Predicted Wmax was based on gender, body weight and height calculated by following formulae:

$$\text{Men: } 25\%\ predicted\ cycling\ Wmax = \frac{(-1{,}78 * age) + (0{,}65 * weight) + (1{,}36 * lenght) - 45{,}4}{4}$$

$$\text{Women: } 25\%\ predicted\ cycling\ Wmax = \frac{(-1{,}19 * age) + (0{,}96 * lenght) + 28{,}1}{4}$$

After six minutes of cycling, subjects remained seated on the bike for an additional six minutes. A second and third exercise bout of six-minute was initiated each interspersed by six-minute recovery (**Figure 6**).

Pulmonary gas exchange was measured continuously breath-by-breath with a mass spectrometer and volume turbine system (Jaeger Oxycon, Erich Jaeger GmbH, Germany). During SM-CPET, VO2 (ml/min), VCO2 (ml/min) and VE (l/min) was assessed breath-by-breath, after which these data were averaged every 10 seconds. Heart rate was continuously monitored by 12-lead ECG device (Kiss, Anandic medical systems INC, Feuerthalen ZH, Switserland). Predicted maximal heart rate was calculated by 220 – age.

Following each exercise bout, capillary blood samples were obtained from the fingertip to analyze blood lactate concentrations (mmol/l) using a portable lactate analyzer (Accutrend Plus®, Roche Diagnostics Limited, Sussex, UK). At the end of each exercise bout, ratings of perceived exertion (RPE) was scored by the patient on a 6-20 Borg scale.

Exercise-onset VO2 kinetics obtained from this test are expressed as O2 deficit and mean response time (MRT) which is calculated by an algebraically method (50). Resting VO2 was calculated as the VO2 during the final minute before exercise. Steady-state VO2 was defined as the average value between the fifth and sixth minute of cycling. The difference between the rest VO2 and steady-state VO2, multiplied by exercise time (six minutes), was defined as the expected amount of VO2 during entire exercise bout.

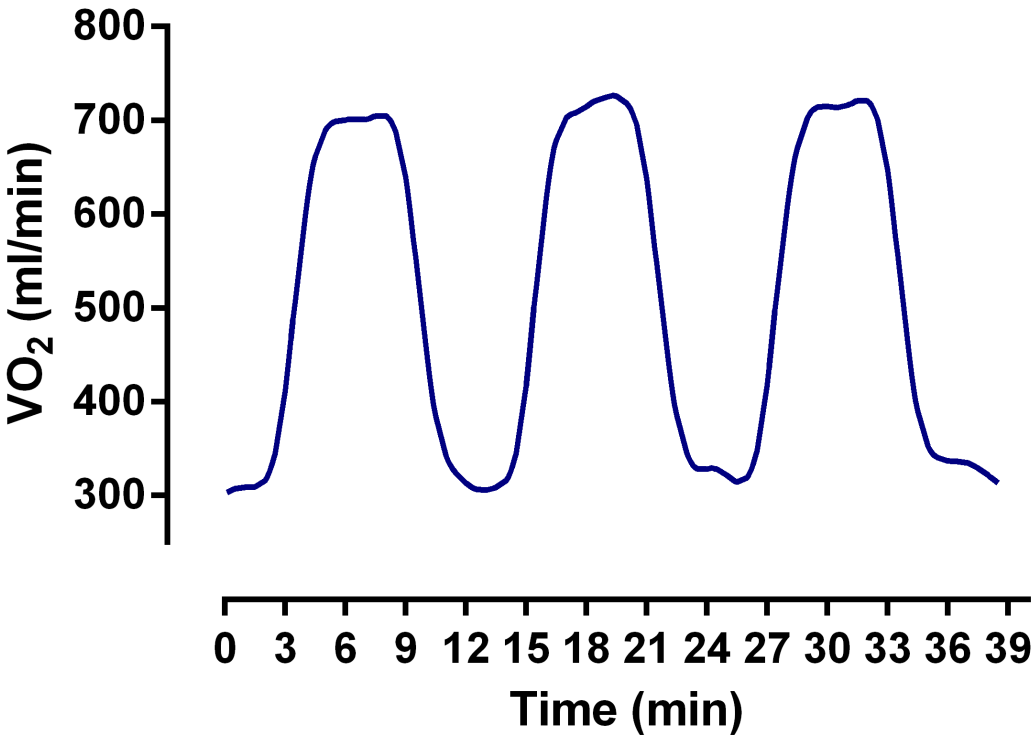

Figure 6: Submaximal cardiopulmonary exercise test (SM-CPET)

To examine skeletal muscle oxidative capacity, cardiodynamic phase of VO2 kinetics were ignored from exercise-onset VO2 kinetics. Therefore, the first 20 seconds of obtained data after onset of exercise were eliminated. The sum of VO2 above resting level was defined as the actually achieved VO2 during entire exercise bout. Oxygen deficit was calculated by: expected amount of VO2 – actually achieved VO2. Division of this oxygen deficit by the difference between rest VO2 and steady-state VO2 equals exercise MRT. The resultant exercise MRT, multiplied by 60, produces a value expressed in seconds. This outcome was used throughout to quantify exercise-onset VO2 kinetics. Finally, the three obtained exercise MRT's, from the three exercise bouts, were averaged.

2.6 Transthoracic echocardiography (TTE)

Vivid 7 portable Ultrasound Machine by GE healthcare (Milwaukee, USA) was used to evaluate the cardiac function of AS patients before and after surgery. Transthoracic echocardiography (TTE) with tissue Doppler was executed by a cardiologist with the patient in the left lateral decubitus position. A GE M4S Matrix Array sector transducer (GE healthcare, Milwaukee, USA) was used to obtain several images of the heart. The TTE protocol included visualization of subcostal four-chamber view, apical four-chamber view, apical two-chamber view and parasternal long-axis and short axis view. Simpson's rule algorithm was used to estimate EF were apical 4 and 2 chamber views were analyzed. Left-ventricular (LV) systolic and diastolic function was assessed using following parameters: ejection fraction (EF), LV septum width (mm), LV diameter (mm), left-atrial (LA) diameter (mm), trans-mitral peak early diastolic velocity (E mitral [cm/sec]), trans-mitral peak late diastolic velocity (A mitral [cm/sec]), mitral E/A ratio, deceleration time (DT [sec]), E/E'- ratio (E'=Early diastolic mitral annular velocity) and cardiac output (CO [l/min]) (51). LV septum width, LV diameter and LA diameter were

assessed on the parasternal long axis (PLAX). E mitral, A mitral, DT and CO, cardiac output index (COi) were evaluated on the apical 4 chamber view. Moreover, pulmonary hypertension (PAPs), AVA, tricuspid annular plane systolic excursion (TAPSE) were also analyzed in patients before and after mini-AVR surgery with TTE.

2.7 Past physical activity

Past physical activity was assessed by the Baecke questionnaire that has been validated for the Dutch elderly population (52, 53). This questionnaire measures a person's habitual physical activity into three distinct dimensions: work activity, sports activity and leisure activity. The average of these three scores represented the past physical activity. The range of physical activity was scored as followed: 1 = < 1 hour; 2 = 1-2 hours; 3 = 2-3 hours; 4 = 3-4 hours; 5 = > 4 hours. Higher scores correspond to higher activity levels.

2.8 Mini-AVR surgery

2.8.1 Anesthetic management

Subjects received standard premedication (Diazepam 10mg, Teva, UK) one hour before arrival to the operating room. Anesthesia was induced with intravenous sufentanyl and propofol and maintained by a combination of propofol (2 -3 mg/kg/h) and isoflurane and muscle relaxation was achieved with pancuronium (0,1 mg/kg). A full dose of heparin (300 IU/kg intravenously) was given and activated clotting time was maintained above 400 seconds. On completion of the procedure, heparin was reversed with protamine at 1:1 equivalent dosage.

2.8.2 Cardiopulmonary bypass

Masuet HL30 heart lung machines (Masueet Cardiopulmonary, Hirrlingen, Germany) was used. Minimal extracorporeal circulation consisted of a totally closed Bioline heparin coated system circuit with rotaflow centrifugal pump, Quadrox-i microporeus membrane oxygenator and venous bubble trap (VBT) (Maquet Cardiopulmonary, Hirrlingen, Germany). A blood collection reservoir connected to the VBT was integrated in the circuit. No open venous reservoir was present. Autologus retrograde priming of the MECC was performed, reducing priming volume to 250 cm^3. Cell saver drainage was used for intrapericardially bloodshed. A pulmonary artery vent (Medtronic Inc, DLP catheter 13 Fr, Minneapolis, USA) was inserted via the main pulmonary trunk distal to the pulmonary valve. Optional sump suction directly through the aortotomy was used when necessary. Pulmonary artery vent was directly connected to the venous bubble trap maintaining the same level of vacuum suction. Aortic root vent runs via a drip chamber and was also connected to the venous bubble trap. Continuous carbon dioxide (CO2) field flooding (6 l/min) was maintained during the entire procedure. Antegrade warm blood cardioplegia (Calafi ore, 1.7 mmol/ml potassium) was administered via the aortic root and repeated every 15 – 20 minutes thereafter selectively via the coronary ostia. Nasopharyngeal temperature was kept at 34 ° C.

2.9 In-hospital rehabilitation

During hospital stay, all patients received similar physiotherapy intervention which started in the intensive care unit (ICU) once the patient was able to cooperate (being awake and extubated). The patients' blood pressure and heart rate were stable, signs of myocardial ischemia were absent, and no malignant cardiac arrhythmias occur. In general, patients remained 1 to 2 days in the ICU. During this intervention, the patient learned deep breathing exercises for a duration of 15 minutes a day and was gradually mobilized. Patients also received a respiratory device for autonomic respiratory exercises during the entire hospital stay, and were advised to execute exercise at least twice a day. When the patient was discharged from the ICU and moved to a regular room, endurance exercises (walking in corridor, walking the stairs and cycling against resistance on ergometer at 60-70 rpm) up to 30 minutes a day at a low intensity (exercise HR <120 beats/min) were added on top of the breathing exercises and leg strength exercises. Patients were carefully monitored for sternal wound healing and/or pain. Unilateral upper extremity exercises were, however, not allowed. The walking distance and cycling resistance were gradually increased, with the aim to mobilize the patient.

2.10 Statistical analysis

The data analyses occurred in several stages and were performed in SPSS (version 24.0, IBM SPSS Inc., Chicago, IL, USA) and GraphPad (Prism 6.01, GraphPad Software, Inc., La Jolla, Ca, USA). First, descriptive statistics were computed and normal distribution of quantitative variables were checked using Shapiro-Wilk test. Most of the data were normally distributed, therefore the data are expressed as mean ± SD.

Normal distributed quantitative variables (CVD, SM-CPET and physical activity) between healthy controls and pre-operative variables of AS patients were analyzed with an independent t-test were a Bonferroni correction was performed. Non-parametric quantitative data between these two groups were tested by Mann-Whitney U test in which Bonferroni corrections were applied (statistical significance was set at $p < 0{,}025$). Next, a two-sided χ^2 test was used for qualitative variables (smoking status and medication use) between healthy controls and AS patients.

Normal distributed quantitative variables (SM-CPET, peri-operative parameters, echocardiography and physical activity) in time were tested with a paired t-test were a Bonferroni correction was performed (pre-operative versus post-operative, n=14). Quantitative variables in time which were not normally distributed, were analyzed using a Wilcoxon signed rank test, in which Holm-Bonferroni corrections for multiple comparisons (pre-operative versus post-operative, n=14) was applied.

Moreover, Pearson correlations and Spearman correlations for continuous data were calculated. A forward stepwise multivariate regression model analyzing the relations between exercise MRT, equivalents of O2 and CO2 and baseline parameters (age, gender, peri-operative parameters, physical activity, medication and CVD) was performed. A final multivariate linear regression was developed were relations between changes in exercise MRT and equivalents of O2 and CO2 and significantly independent predictors that were found from the forward stepwise multivariate regression were determined. The statistical significance in these regression models was set at $p<0{,}05$ (2-tailed).

Furthermore, an interim analysis was performed to calculate the observed statistical power (G-power, 3.1.9.2, Düsseldorf, Germany). Observed power of this observational study was calculated for EqO2: α = 0,808; EqCO2: α = 0,784 and exercise MRT: α = 0,621.

3. Results

3.1 Study population

In this prospective observational study, 14 patients with severe AS who received a new aortic valve via mini-AVR surgery, were fully age-, gender- and BMI matched with 14 healthy controls free of any chronic diseases. Subjects baseline characteristics and descriptive data are summarized in table 1.

Table 1: Descriptive data and characteristics of healthy controls and AS patients

Variable	Healthy controls	AS patients
Subjects (n)	14	14
Male	6	6
Female	8	8
Age (years)	66,7 ± 12,7	67,1 ± 12,5
Height (cm)	163,4 ± 9,1	163,4 ± 9,7
Weight (kg)	68,5 ± 9,8	70,9 ± 10,8
Body Mass Index (kg/m^2)	25,7 ± 3,4	26,6 ± 4,1
Systolic blood pressure (mmHg)	132,8 ± 15,9	143,6 ± 22,7
Diastolic blood pressure (mmHg)	**80,7 ± 9,16***	79,3 ± 12,7
Waist circumference (cm)	**91,1 ± 10,9***	88,5 ± 17,0
Past physical activity (hours/week)	3,1 ± 1,4	2,4 ± 0,6
Bicuspid aortic valve (n)	--	1
Medication (n)		
Anticoagulants	2	8
Beta-blockers	0	2
Anti-hypertensive agents	2	6
Antiarrhythmic agents	0	1
Diuretics	0	4
Statins	3	6
Antibiotics	0	1
Analgesic	0	1
Inflammatory inhibitors	0	1
Others	2	7
Cardiovascular disease risk		
Obesity (BMI ≥ 30 kg/m^2, n)	1	4
Diabetes (metformin, n)	0	2
Hypertension (≥140/90mmHg, n)	3	12
Smoking (n)	2	3

Values are represented as mean±SD. *Significant difference between healthy controls and patients in need of mini-AVR surgery ($p < 0,05$). Patients taking blood pressure lowering drugs are considered hypertensive.

AS patients were aged 44-83 years (67,1 ± 12,5) of which 43% were men. Focusing on the characteristics between healthy controls and AS patients, no significant difference at baseline were found, with the exception of diastolic blood pressure and waist circumference (**Table 1**). Following medications were taken before surgery by AS patients: anticoagulants (n=8), beta-blockers (n=2), anti-hypertensive agents (n=6), anti-arrhythmic agents (n=1), diuretics (n=4), statins (n=6), antibiotics (n=1), analgesic (n=1), anti-inflammatory agents (n=1) and other medications (n=7) which include benzodiazepines, metformin, thyroid hormones, etc. Medication use in healthy controls consisted of anticoagulants (n=2), anti-hypertensive agents (n= 2) and statins (n=3).

Four of the AS patients were overweight (BMI ≥ 30 kg/m^2) in comparison to 1 healthy control and two AS patients had type II diabetes. Furthermore, three patients with AS smoked actively before mini-AVR surgery.

3.2 Functional capacity of AS patients versus healthy controls

3.2.1 Exercise physiology during a constant-load SM-CPET in AS patients versus healthy controls

Resting VO2 was slightly higher in patients with severe AS during the first and third bout, but no significant difference was observed (321,9 ± 18,7 resp. 295,6 ±45,4; p = 0,599). Steady-state VO2 kinetics (during fifth and sixth min of each exercise bout) during exercise was significantly lower in AS patients versus healthy controls (709,6 ± 95,7 ml/min resp. 847,3 ± 103,4 ml/min; p = 0,001) (**Figure 7**).

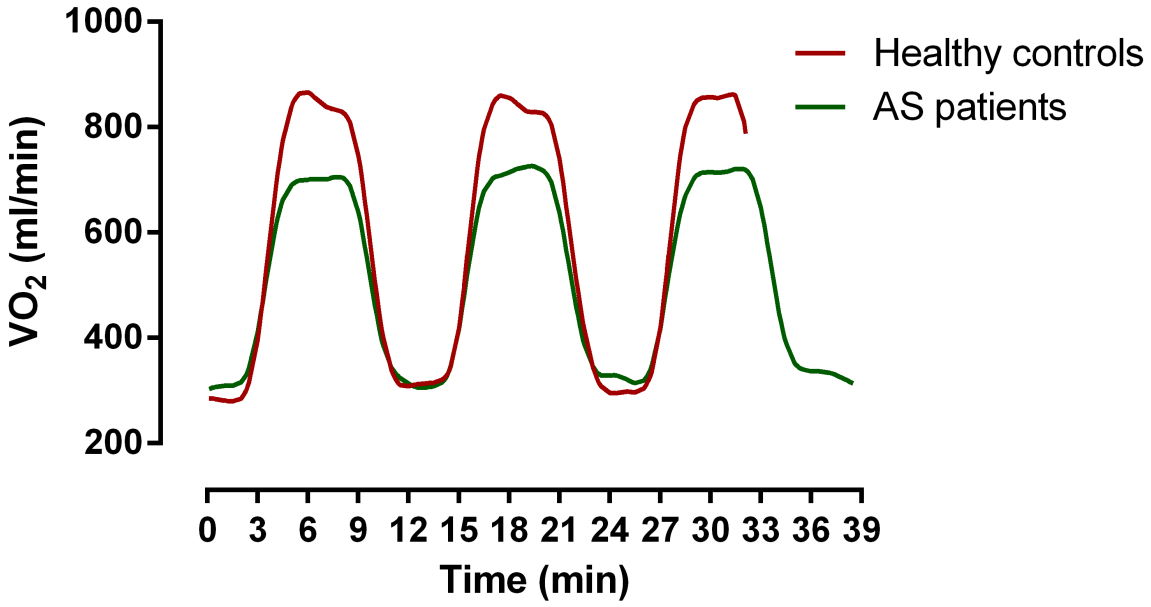

Figure 7: Oxygen uptake (VO2) of healthy controls and AS patients
VO2 at steady-state in AS patients is significantly lower (p = 0,001) vs healthy controls. Red: VO2 kinetics of healthy controls (n=14); green: VO2 of AS patients (n=14) before surgery.

Table 2 shows the SM-CPET exercise test parameters of healthy controls versus AS patients. No significant difference was noticed when cycling power output was compared between healthy controls and patients with AS (26,4 ± 5,7 W resp. 25,7 ± 6,2 W; p = 0,157). Average RER during 3 exercise bouts was significantly higher in healthy controls versus AS patients (p = 0,002). Furthermore, a

significant lowered O2 pulse during exercise was noticed in AS patients compared to healthy controls (p = 0,038). Equivalents for O2 and CO2 during exercise were significantly higher (p = 0,002 resp. p = 0,008) in AS patients versus healthy controls (**Table 4**).

Table 2: Submaximal cardiopulmonary exercise test parameters of healthy controls versus AS patients

Variable	Healthy controls	AS patients	p-value
Cycling power output (W)	26,4 ± 5,7	25,7 ± 6,20	0,157
RER	0,86 ± 0,1	0,91 ± 0,04	**0,002**
Lactate (mM)	03,2 ± 0,70	3,1 ± 1,7	0,751
Borg scale	9,1 ± 1,5	9,6 ± 2,5	0,782
Heart rate (beats/min)	92,3 ± 8,70	87,7 ± 11,6	0,262
Predicted heart rate (%)	59,8 ± 9,5	57,5 ± 7,2	0,489
VO2 (mL/min)	847,3 ± 103,4	709,6 ± 95,7	**0,001**
VCO2 (mL/min)	730,3 ± 107,2	654,2 ± 104,9	0,682
O2 pulse (ml/beat)	9,27 ± 1,5	8,15 ± 1,13	**0,038**
VE (L/min)	21,5 ± 3,7	22,3 ± 3,0	0,497
Equivalent O2	25,4 ± 2,9	31,7 ± 4,7	**0,002**
Equivalent CO2	31,2 ± 8,9	34,6 ± 5,7	**0,008**
VT (mL/min)	1119,9 ± 220,6	1042,2 ± 381,5	0,363

Values are represented as mean±SD. RER: respiratory exchange ratio; VO2: Oxygen uptake; VCO2: Carbon dioxide production; VE: minute ventilation; VT: Tidal volume. Significant difference (p <0,05).

3.2.2 Oxygen uptake kinetics (MRT) in AS patients versus healthy controls

Between groups, exercise MRT was not significantly different (51,1 ± 11,5 sec (healthy controls) compared to 53,1 ± 19,9 sec (AS patients); p = 0,817).

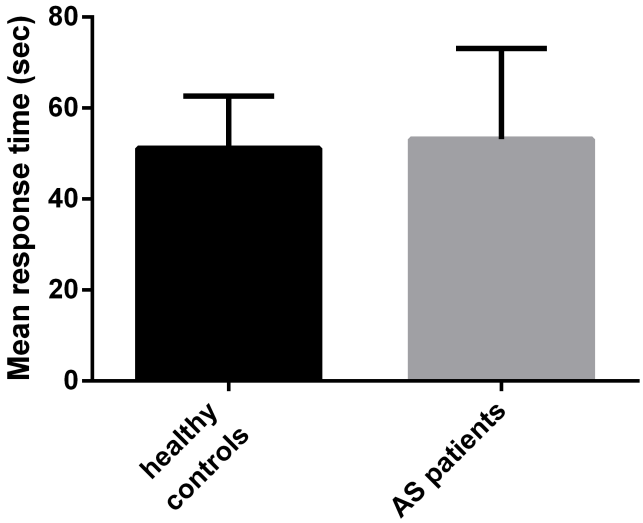

Figure 8: Mean response time (MRT) in AS patients versus healthy controls
No significant difference in exercise MRT during exercise was noticed (p = 0,871); AS: aortic stenosis

3.3 Functional capacity of AS patients at five days after mini-AVR surgery.

3.3.1 Peri-operative parameters during mini-AVR surgery

During mini-AVR surgery, the average aortic cross-clamp time was around 35,3 ± 8,1 minutes and patients were connected to the heart lung machine for a total of 50,5 ± 9,02 minutes. One patient received a mechanical valve, while the other 13 patients received a biological valve. During and in early hours (12-24h) after mini-AVR surgery, patients lost blood due to bleedings which was drained and collected in drainage canisters. After mini-AVR surgery, patients were taken to the ICU where they stayed for an average period of 36,1 ± 15,2 hours. Patients were intubated for a total time of 7,4 ± 4,6 hours and were hospitalized for 6,3 ± 1,6 days (**Table 3**).

Table 3: Peri-operative and early post-operative parameters of AS patients

Variable	Peri-operative parameters
Cardiopulmonary bypass time (min)	50,5 ± 9,02
Cross clamp time (min)	35,3 ± 8,1
Intubation time (hours)	7,4 ± 4,6
Length stay in ICU (hours)	36,1 ± 15,2
Bleeding during surgery (mL)	147,1 ± 214,7
Bleeding 12 hours post-operative (mL)	190 ± 192
Bleeding 24 hours post-operative (mL)	271,1 ± 211,8
Hospitalization (days)	6,29 ± 1,59

ICU: Intensive care unit; Values are represented as mean±SD.

3.3.2 Cardiovascular disease risk of AS patients five days after mini-AVR surgery

Table 4 shows that there was no significant change in cardiovascular disease risk within the AS patients after five days.

Table 4: Characteristics of patients before and after AS surgery

Variable	Pre-operative	Post-operative day 5	Post-operative week 3
Subjects (n)	14	9	9
Weight (kg)	70,9 ± 10,8	71,8 ± 11,9	67,7 ± 8,7
BMI (kg/m^2)	26,7 ± 4,11	25,7 ± 3,23	25,0 ± 3,2
Systolic BP (mmHg)	143,6 ± 22,7	127,7 ± 18,6	12,6 ± 1,3
Diastolic BP (mmHg)	79,3 ± 12,7	74,4 ± 7,3	7,2 ± 0,7
Waist circumference (cm)	88,5 ± 17,0	91,4 ± 9,75	87,2 ± 9,5

Values are represented as mean±SD. BP: Blood pressure;

3.3.3 Exercise physiology of AS patients five days after mini-AVR surgery

A higher VO2 at rest at five days after surgery (333,2 ± 50,5 ml/min) compared to pre-operative rest VO2 (322,6 ± 63,1 ml/min) was noticed during the three bouts but VO2 at steady-state remained the same (**Figure 9**).

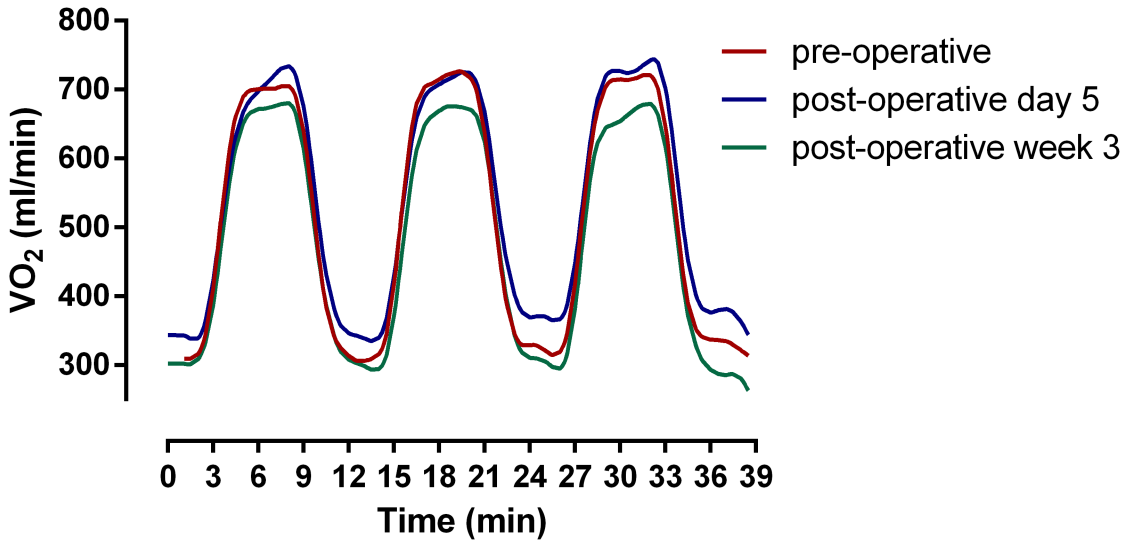

Figure 9: Exercise oxygen uptake (VO2) of AS patients before and after mini-AVR surgery.
VO2 worsen at post-operative day 5 and week 3 in comparison prior to mini-AVR surgery. The average of VO2 before the start of each bout is increased at post-operative day 5 (333,2 ± 50,5 ml/min) in comparison to the average VO2 before the start of each bout (322,6 ± 63,1 ml/min) before mini-AVR surgery took place. No significant differences between VO2 during steady-state were noticed at five days and three weeks after mini-AVR surgery.

At day five after surgery, a significant increase in ratings of perceived exertion during exercise (p = 0,010) was noticed. These patients had a significant higher minute ventilation (VE) (p= 0,007) and significantly higher equivalents of O2 and CO2 during exercise (p = 0,008). Also, tidal volume (VT) was significantly lower at five days during exercise after mini-AVR surgery (p = 0,009) (**Table 6**). The three patients who were unable to complete their first bout of exercise, had even higher average equivalents of O2 and CO2 at rest (EqO2 = 39,26 ± 6,5 ml/min; EqCO2 = 49,45 ± 7,1 ml/min).

3.3.4 Exercise kinetics in AS patients five days after mini-AVR surgery

Exercise MRT was significantly increased five days after mini-AVR surgery (65,2 ± 12,5 sec) compared to pre-operative exercise MRT (53,1 ± 19,9 sec) in AS patients (p = 0,036) (**Figure 10**).

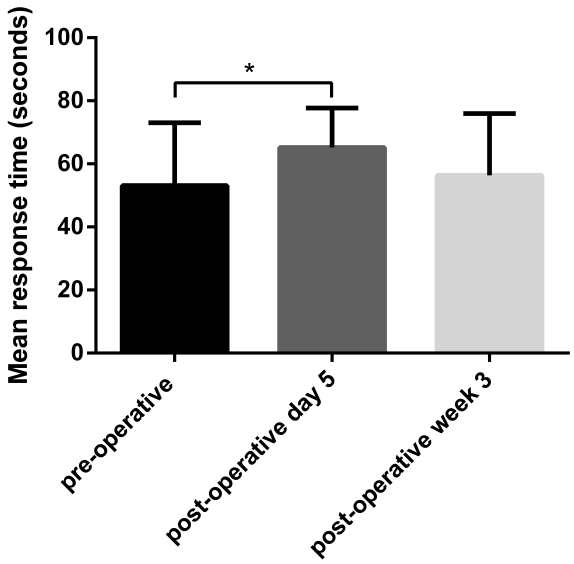

Figure 10: Exercise mean response time (MRT) of AS patients early after surgery
A significant increase ($p<0,05$) in exercise MRT was only observed five days after mini-AVR surgery. Three weeks after surgery the exercise MRT was decreased but still increased compared to the pre-operative status in AS patients

3.4 Functional capacity of AS patients at three weeks after mini-AVR.

3.4.1 Cardiovascular disease risk of AS patients at three weeks after mini-AVR surgery

Table 4 shows that there was also no significant change in cardiovascular disease risk at three weeks post-surgery.

3.4.2 Exercise physiology of AS patients three weeks after mini-AVR surgery

Figure 9 shows lowered steady-state VO2 during exercise compared to five days after mini-AVR surgery. A significant lower heart rate during exercise was observed ($p = 0,012$) at this time point. Equivalent of CO2 (36,9 ± 5,5 ml/min) during exercise decreased significantly at week three ($p = 0,043$ resp. $p = 0,010$) and were normalized as opposed to the equivalents prior to mini-AVR surgery. A trend in lowered equivalents of O2 ($p = 0,051$) was also observed at three weeks (**Table 5**).

3.4.3 Exercise kinetics in AS patients three weeks after mini-AVR surgery

Exercise MRT (56,4 ± 19,6 sec) was accelerated versus five days after mini-AVR surgery (65,2 ± 12,5 sec; $p = 0,149$) and was normalized opposed to exercise MRT before surgery ($p = 0,503$) (**Figure 10**).

3.4.4 Transthoracic echocardiography (TTE)

Three weeks post-surgery, most of the patients had at least minimal pleural congestion. In some patients, diuretics were started to prevent further pulmonary congestion. Further analysis of several TTE variables is needed to obtain quantitative data to evaluate possible significant differences between the cardiac function prior to surgery and early after surgery (within three weeks).

Table 5: Submaximal cardiopulmonary exercise test parameters of AS patients prior and post AS patients.

Variable	Pre-operative	Post-operative day 5	p-value 1	Post-operative week 3	p-value 2	p-value 3
Cycling power output (W)	25,7 ± 6,20	--	--	--	--	--
RER	0,91 ± 0,04	0,92 ± 0,06	0,772	0,93 ± 0,05	0,594	0,028
Lactate (mM)	3,1 ± 1,7	3,1 ± 0,7	0,672	2,9 ± 0,9	0,310	0,757
Borg scale	9,6 ± 2,5	11,3 ± 2,4	**0,010**	10,7 ± 2,6	0,110	0,216
Heart rate (Beats/min)	87,7 ± 11,6	88,9 ± 8,0	0,284	82,5 ± 18,7	0,466	**0,012**
Predicted heart rate (%)	57,5 ± 7,20	56,9 ± 4,90	0,314	52,9 ± 13,9	0,173	0,063
VO2 (mL/min)	709,6 ± 95,7	713,3 ± 56,0	0,313	675,6 ± 97,3	0,151	0,244
VCO2 (mL/min)	654,2 ± 104,90	658,2 ± 76,7	0,419	633,2 ± 122,9	0,210	0,770
O2 pulse	8,15 ± 1,13	8,05 ± 0,62	0,816	8,41 ± 1,50	0,142	0,055
VE (L/min)	22,3 ± 3,0	27,6 ± 5,8	**0,007**	23,4 ± 5,80	0,616	0,047
Equivalent O2	31,7 ± 4,7	38,7 ± 8,3	**0,008**	34,3 ± 5,10	0,051	0,043
Equivalent CO2	34,6 ± 5,7	41,8 ± 6,9	**0,008**	36,9 ± 5,50	**0,021**	**0,010**
VT (mL/min)	1042,2 ± 381,5	1040,6 ± 194,9	**0,009**	931,1 ± 234,1	0,038	0,326

Values are the average of the submaximal exercise test consisted of three bouts and are presented as mean±SD. RER: respiratory exchange ratio; VO2: Oxygen uptake; VCO2: Carbon dioxide production; VE: minute ventilation; VT: Tidal volume; Significant difference ($p < 0,017$; $p < 0,025$; $p < 0,05$) was corrected for Holms-Bonferroni. P-value 1: p-value between pre-operative and five days after mini-AVR surgery. P-value 2: p-value between pre-operative and three weeks after mini-AVR surgery. P-value 3: p-value between five days and three weeks after mini-AVR surgery

3.5 Univariate correlations

A positive significant correlation was found between ratings of perceived exertion and equivalents of O2 during exercise before mini-AVR surgery (r = 0,543; p=0,045) (**Figure 11A**). Pre-operative equivalents of O2 were negatively correlated with heart rate during exercise (r = -0,554; p = 0,04). Furthermore, equivalents of CO2 were positively correlated with age (r = 0,629; p =0,016) (**Figure 11B**). A correlation was found with exercise MRT (r = 0,588, p = 0,035) and Borg scale.

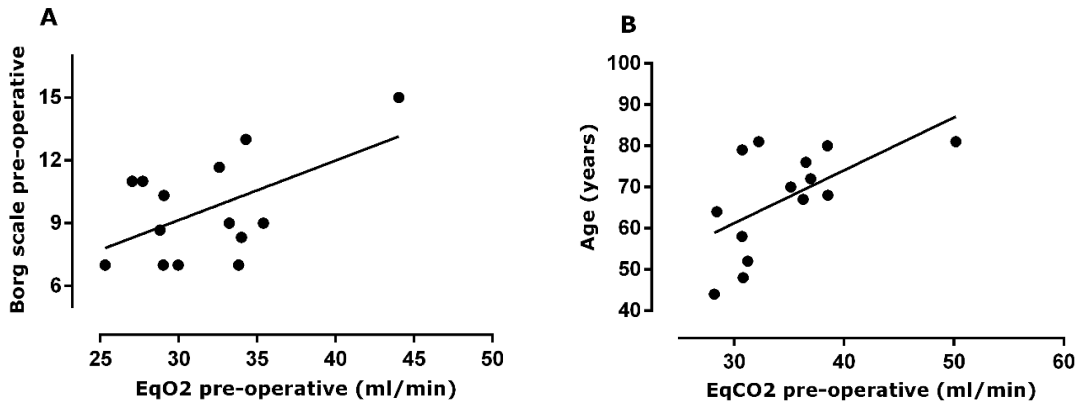

Figure 11:Univariate correlations prior to mini-AVR surgery
Correlations were significant at the 0,05 level (2-tailed). Positive correlation between equivalent of O2 and Borg scale in AS patients was found before mini-AVR. Furthermore, equivalent of CO2 was correlated with the age of AS patients.

After five days a positive correlation was found between O2 equivalents and the cross-clamp time during mini-AVR surgery (r = 0,669; p = 0,049) (**Figure 12**).

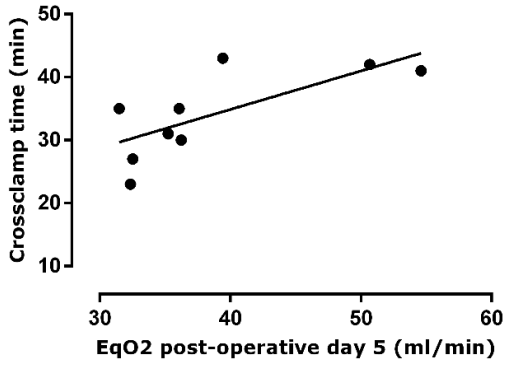

Figure 12: Univariate correlations at five days after mini-AVR surgery
Correlations were significant at the 0,05 level (2-tailed).

Cross-clamp time (r = 0,843; p = 0,004) (**Figure 13A**) and time connected to the heart lung machine (bypass time) (r = 0,716; p = 0,03) (**Figure 13B**) strongly correlated with exercise MRT three weeks after mini-AVR surgery.

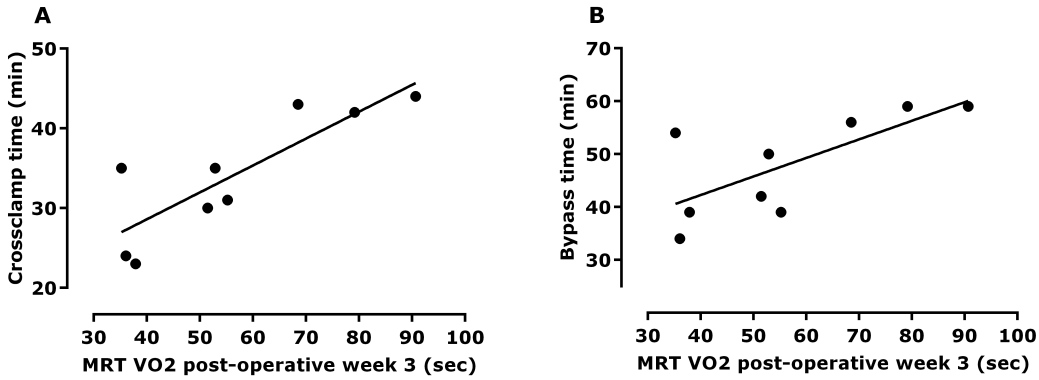

Figure 13: Univariate correlations at three weeks after surgery.
Correlations were significant at the 0,05 level (2-tailed).

3.6 Regression analysis

Longer cross-clamp time during mini-AVR was independently related (R^2 = 0,935; β = 0,701; p =0,001) with an accelerated exercised MRT at three weeks after surgery during exercise.

4. Discussion

In this present observational study, the impact of mini-AVR surgery on cardiopulmonary- and oxidative muscular function during exercise was investigated in order to demonstrate the effect of this new surgical approach on the patient. A worsening in equivalent of O2 (EqO2) and CO2 (EqCO2) during exercise of AS patients was observed compared to healthy controls. The main finding of this study was a worsening in EqO2 and EqCO2 during exercise early after mini-AVR surgery due to possible ventilation-perfusion mismatch. Furthermore, exercise VO2 kinetics during exercise were slowed early (five days) after mini-AVR surgery.

In order to assess exercise physiology in AS patients, a submaximal exercise test was performed. It was found that steady state VO2 during exercise was significantly lower in AS patients. A possible reason for this decrease is a compromised cardiac output (CO) due to a stenotic aortic valve. It is known that VO2 is dependent from the ability of the heart to pump out blood (54). In line with this findings, Dhoble et al. demonstrated a lowered peak VO2 during maximal exercise test in a small population of patients with AS (55). Despite that a max-CPET was conducted in the study of Dhoble *et al*., the same conclusion could be drawn. During SM-CPET, AS patients had a significant lowered O2-pulse during exercise compared to healthy controls which may indicate a lower cardiac stroke volume. A possible reason for a decreased stroke volume is a reduced velocity of fiber shortening by a raise in afterload in AS patients. Left ventricular emptying is impaired in AS due to higher outflow resistance which causes a large pressure gradient across the aortic valve during ejection. This has an increased effect on peak systolic pressure leading to an increased afterload which results in a decreased stroke volume (56). In addition, AS patients had significantly elevated equivalents of O2 (VE/VO2) ($p = 0,002$) and CO2 (VE/VCO2) ($p = 0,008$) during exercise, when compared to healthy controls. A possible suggestion for the higher equivalent values is that there is a possible decrease in gas exchange efficiency which implies a possible higher ventilation-perfusion mismatch in these patients. Ventilation-perfusion ratio is the amount of air reaching the alveoli (alveolar ventilation) and the amount of blood sent to the lungs (CO). Since CO is impaired in AS patients, a lower amount of blood reaches the lungs (decrease in perfusion) and AS patients had a slightly increased ventilation (VE) leading to an increased ventilation perfusion ratio (57). Moreover, higher equivalents of O2 and CO2 reflect ventilatory performance which means that AS patients have a compensatory increased ventilation due to cardiovascular limitations (38). EqO2 during exercise was also significantly correlated with Borg scale. This means that with increased Eq02, AS patients are more prone to experience ratings of perceived exertion. A possible explanation could be that due to a compromised CO during exercise, an increased in Eq02 is observed in order to keep the blood from becoming too acidic because of high CO2 levels. Higher EqO2 during exercise lead to higher VE to try to meet O2 demand which results in faster ratings of perceived exertion. Furthermore, a positive correlation was found between EqCO2 and age during exercise. Older AS patients have higher EqCO2 which means that a decrease in ventilatory efficiency is observed. These results are in line with the study of Forman *et al*. who saw that age in heart failure patients correlated strongly with EqCO2 during exercise (58). In this present study no significant difference ($p > 0,05$) was observed in oxygen uptake (VO2) kinetics during exercise, expressed as mean response time (MRT), between AS patients and healthy

controls which means that the AS patient population have similar exercise MRT. Exercise MRT was not sensitive enough to detect any abnormalities in AS patients prior to surgery.

Five days after mini-AVR surgery, no changes were observed in O2 pulse during exercise ($p = 0,038$). This suggest that no improvement in cardiac stroke volume was observed early after mini-AVR surgery, implying that cardiac function after five days is not improved. A significant increase in Borg scale after exercise was observed among the AS patients. Because AS patients received a new aortic valve, it was expected that no increase in ratings of perceived exertion due to an increased blood outflow and an improved cardiac function could be observed. In contrast to this hypothesis, the findings of this study showed the opposite. A possible explanation for increased ratings of perceived exertion early after mini-AVR (5 days) are the significantly increased EqO2 ($p = 0,008$) and EqCO2 ($p = 0,008$) during exercise due to a possible worsened ventilation-perfusion mismatch. This can possibly be explained by systemic inflammatory response syndrome (SIRs) after mini-AVR. AVR under cardiopulmonary bypass can cause a significant elevation in plasma cytokine levels due to surgical trauma which can provoke inflammation in different organs resulting in possible respiratory failure (59). It is well known that inflammation occurs after cardiac surgery (60), but SIRs criteria have not been investigated in patients undergoing mini-AVR. Because the lack of SIRs criteria, inflammatory response in AS patients is unknown (24). A second possible cause is the presence of subclinical pulmonary embolisms after mini-AVR surgery. To eliminate this possible cause, D-dimer determination has to be performed five days after mini-AVR. However, it has already been shown by Josa et al. that pulmonary embolism did not develop in any patient who had isolated mini-AVR surgery (61). A third possible reason is that pulmonary edema after mini-AVR surgery can occur, which decreases the ventilatory function resulting in higher EqO2 and EqCO2 during exercise and worsen ventilation-perfusion mismatch (62). A fourth, more plausible cause is overfilling of the heart. Overfilling of the heart by volume infusion after mini-AVR surgery can lead to ventricular distention, pulmonary edema and worsening of myocardial performance (63). Further analysis of several TTE parameters is needed to confirm the hypothesis that worsen EqO2 and EqCO2 during exercise are caused by overfilling of the heart after mini-AVR surgery. Furthermore, five patients were unable to complete exercise test at five days after surgery due to rating of perceived exertion and had even higher resting EqO2 and EqCO2. A possible reason why these patients were unable to perform any physical activity remains unknown. Therefore, further investigation is needed to find underlying mechanisms.

After five days, a significant correlation was found between EqO2 during exercise and cross-clamp time ($r = 0,669$; $p = 0,049$) during mini-AVR surgery. Patients have a higher ventilation rate than oxygen consumption during exercise with increasing cross-clamp time during surgery. A possible reason for an increased EqO2 with increasing cross-clamp time, is that the gas exchange at the level of the lungs is not efficient due to pulmonary dysfunction probably caused by edema. Paterson *et al*. showed that a prolonged aortic cross-clamp time was associated with increased pulmonary arterial pressure, and higher pulmonary vascular resistance. Moreover, in his study pulmonary edema was observed in all patients and with increasing aortic cross-clamping, pulmonary dysfunction was manifested (64). After five days, exercise MRT was significantly slower than before mini-AVR surgery ($p = 0,036$). Slowed MRT during submaximal exercise was identified by Hansen et al. in multiple sclerosis patients. In this study, it was suggested that this was due to low oxidative muscle capacity

(65). Initially in AS patients it was hypothesized that worsened exercise MRT could be a result of a peripheral malfunction, but no changes in oxidative muscle functions and lactate were observed after five days. From these findings, it can be concluded that underlying etiology of worsened exercise MRT lays probably with a central malfunction. Because no significant and independent relationships were found between exercise MRT at five days after surgery and peri-operative parameters as well as exercise physiology variables, it is difficult to explain why exercise MRT is slowed. Further research is needed to investigate underlying ethology causing increased exercise MRT early after mini-AVR surgery.

At three weeks after mini-AVR surgery, a trend in higher O2-pulse was noticed. It was expected that after three weeks O2-pulse would have reached normal values and be significantly different because of a new aortic valve. This implicates that the left ventricle (LV) systolic performance is not significantly improved compared to LV systolic performance before surgery. This indicates no improvement in cardiac function after three weeks. This finding was in contrast to the findings showed in the study of Tonga *et al*. were they found that O2-pulse was significantly increased after mitral valve repair (66). After three weeks significantly lower EqCO2 ($p = 0,021$) at three weeks compared to five days after mini-AVR surgery were observed. This implicates that ventilation efficiency is improved compared to five days after mini-AVR, but not improved to the patient's levels prior to mini-AVR surgery. This indicates that at three weeks after mini-AVR surgery, a possible ventilation-perfusion mismatch is still present. This can be explained by a significant lowered VE indicating a better ventilation efficiency at three weeks compared to five days. These findings indicate no significant improvement in pulmonary function early after mini-AVR surgery.
After three weeks, exercise MRT was accelerated compared to five days but no significant difference was observed and was normalized when compared to exercise MRT prior to mini-AVR surgery. The slowed exercise MRT could possibly be explained by a lowered VE and EqCO2 suggesting a possible improved ventilation efficiency after three weeks. It remains difficult to explain these findings whereby further investigation is needed. Exercise MRT was strongly correlated with cardiopulmonary bypass time ($r = 0,716$; $p = 0,03$) and aortic cross-clamp time ($r = 0,843$; $p= 0,004$) at three weeks. The longer AS patients were connected to the heart lung machine and aortic clamp, the higher MRT during exercise. A possible suggestion is that AS patients who had an increased cardiopulmonary bypass time and cross-clamp time, have an increased risk of ventilatory dysfunction. This could lead to a decreased O2 uptake by the lungs which leads to a delayed O2 uptake and consumption by skeletal muscles which results in slowed exercise MRT (64). Cross-clamp time is a strong independent predictor of exercise MRT at three weeks after mini-AVR surgery. But, further investigations are needed to find the actual underlying etiology.

5. Conclusion

This prospective study showed that early after mini-AVR surgery, a systematic significant deterioration in exercise performance was observed which is probably related to ventilation perfusion mismatch. Need for improved early post-operative treatment, within days, is of great importance and necessary to prevent further worsening of physical condition of these patients.

6. Future prospective

A total of 17 AS patients has to be included to achieve a power of 0,80 in both EqCO2 and exercise MRT. Future studies are required to investigate underlying etiology causing the possible worsened ventilation-perfusion mismatch. Therefore, physicians and cardiothoracic surgeons have to search for a possible improved early post-operative treatment. In addition, the effect of a three-month rehabilitation on physical condition of AS patients has to be investigated. At six months after mini-AVR surgery, AS patients will receive a SM-CPET, CVD risk assessment and echocardiography to determine the effect of rehabilitation on cardiopulmonary and oxidative muscular function.

7. Limitations of this study

This prospective study had several limitations. Because of the elderly population, it was difficult to find gender and BMI matched healthy controls. These healthy controls did not all receive a TTE. Therefore, we could not be 100% sure that these healthy controls did not have aortic stenosis.
Further, it was anticipated to measure present physical activity during entire hospital stay by accelerometry. Patients were also asked to wear the accelerometer during a week starting from the time point at three weeks after surgery. Unfortunately, AS patients did not wear this device consequently by which no results of present physical activity after mini-AVR surgery could be obtained. Another limitation was that TTE images were not analyzed in time, which lead to a lack of important information about cardiac function after mini-AVR surgery.

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