Validity and Prognostic Value of Cardiac Output measurement using the $\text{CO}_2$ -rebreathing technique during exercise in children with Congenital Heart Defects

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Dissertation presented in the 2nd Master year in the program of Master in Medicine in Medicine
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ACKNOWLEDGEMENTS

When we started this research paper, we knew this would be a challenging project. There were many things about the subject that we didn’t know, or couldn’t even begin to understand. We started reading a lot about the topic and got more and more enthusiastic and curious. Eventually, after many ups and downs we got to the bottom of it. In the end we got so involved with this project that it was hard to let it go and be able to round up this project.

We would like to thank our co-promotor dr. Kristof Vandekerckhove for his very adequate guidance, to always answer our emails within a day or two with an adequate answer to our problems, for his unremitting patience when we had a hard time understanding his difficult explanations. We would also like to thank him for his support and encouragement, and for challenging us to go further that we normally would. We couldn’t have asked for a better promotor.

We would like to express a special thanks to Ir. Ilse Coomans, technician of the pediatric cardiology. She invited us to attend a couple of exercise tests, she made it a point of honor to explain us the exact operation of the used equipment. She also provided us with valuable statistical advice, but above all she motivated us with her characteristic contagious enthusiasm about the project.

We would like to thank each other for the easy and fluent cooperation during the last two years. It was pleasant to work together because we consistently kept to the agreed plan, and we both continuously lived up to the expectations we had for each other. We complemented one another throughout the whole process; we often had our differences in opinion, but we always succeeded to end up with the best possible outcome.

Finally, we would like to thank our friends and family for their support throughout the whole process.
CONTENT

Abstract ......................................................................................................................................... 1

Introduction .................................................................................................................................... 2

1. Cardiac output definition and value ......................................................................................... 2

2. Use of spiroergometry in CPET: definition and method......................................................... 5
   2.1 Ergometry .................................................................................................................................. 6
   2.2 Spirometry ................................................................................................................................. 6
   2.3 Maximal Effort ......................................................................................................................... 10

3. Different cardiac output measurement methods ..................................................................... 12
   3.1 Invasive ..................................................................................................................................... 12
      3.1.1 Pulse Dye Densitometry ...................................................................................................... 12
      3.1.2 Transpulmonary Thermodilution ....................................................................................... 13
      3.1.3 Right Heart Catheterization ............................................................................................... 13
      3.1.4 O₂ rebreathing with Direct Fick .......................................................................................... 14
   3.2 Noninvasive ............................................................................................................................ 15
      3.2.1 CO₂-rebreathing with Indirect Fick ................................................................................... 15
      3.2.2 Inert Gas Rebreathing ......................................................................................................... 18
      3.2.3 Doppler Echocardiography ............................................................................................... 19
      3.2.4 Electrical Impedance Cardiography .................................................................................. 19
      3.2.5 Other methods .................................................................................................................... 20

Methods .......................................................................................................................................... 20

1. Literature research .................................................................................................................... 20

2. Study upset ............................................................................................................................... 21
   2.1 Study Subjects ......................................................................................................................... 22
   2.2 Data collection .......................................................................................................................... 24
   2.3 Equipment ............................................................................................................................... 24

3. Statistical Analysis .................................................................................................................... 25
Results ................................................................................................................................................. 26

1. Assessment of reliability of CO measurement .............................................................................. 26

2. Comparison of characteristics between Congenital Heart Defects and control group ........ 27

   2.1 Coarctatio .................................................................................................................................. 28
   2.2 Univentricular ........................................................................................................................... 29
   2.3 Tetralogy of Fallot ..................................................................................................................... 30
   2.4 Transposition of the great arteries ......................................................................................... 31

Discussion .......................................................................................................................................... 32

1. Correlation of CO in the control group ......................................................................................... 33

2. Comparison of heart patients with the control group ................................................................. 35

   2.1 Coarctatio .................................................................................................................................. 35
   2.2 Univentricular ........................................................................................................................... 35
   2.3 Tetralogy of Fallot ..................................................................................................................... 36
   2.4 Transposition of the great arteries ......................................................................................... 38

3. Study Limitations ......................................................................................................................... 40

4. Future Perspectives ....................................................................................................................... 41

Conclusion .......................................................................................................................................... 41

References ........................................................................................................................................... 43
ABSTRACT

Objective: Cardiac output measurement, as a pure hemodynamic parameter, could be a useful tool for follow up of children with congenital heart defects. Noninvasive measurement of cardiac output during exercise might be the most adequate method for evaluation of exercise capacity.

Method: In this retrospective study, we analyzed exercise tests of patients with congenital heart defects, as well as children with normal structured hearts. Each subject performed a CPET on cycle ergometer. Before and after each test, cardiac output was measured using the CO₂-rebreathing method with indirect Fick, so resting values and values at maximal intensity were obtained.

Results: There was a strong positive correlation (r=0.678, p<0.001) between CO₉max and VO₂max within the control population. Patients for the univentricular group had the strongest statistically significant (p<0.001) difference for CO₉max compared to the control group. There wasn’t a statistically significant difference (p=0.789 resp. p=0.069) for CO₉max respectively for TOF and TGA. The coarctatio population scored statistically significant (p=0.036) better than the control group.

Conclusion: CO₂-rebreathing with Indirect Fick method is a representable way to measure CO at maximal intensity. It reflects the severity of the CHD, and the limitation of exercise capacity.
INTRODUCTION

1. CARDIAC OUTPUT DEFINITION AND VALUE

Cardiac Output (CO) is a parameter used to determine the left ventricular function. Essentially, it is the blood volume that is pumped from the left ventricle in one minute during the systolic phase. Blood transportation is needed to fulfill oxygen tissue requirements. Hence, during exercise, several cardiovascular mechanisms are forced to increase the delivery of oxygen to the muscles. To fully exploit the maximal capacity of the heart to deliver blood to the exercising muscles, cardiac output is increased. Initially, stroke volume will be raised, but when the stroke volume no longer sufficiently supplies the demanded amount of oxygen to the muscles, the heart rate will increase (1). When there is no intra-cardiac shunt, it is safe to assume that the output from left and right heart are the same, therefore the pulmonary circulation can be considered to have equal blood flow as the systemic circulation assuming that they are connected in series (1). Measurement of CO is a good parameter when trying to determine whether reduced O\(_2\) uptake is due to deficit O\(_2\) transport or failure of the muscles to extract O\(_2\) (2).

CO can be expressed as the product of stroke volume (SV) and heart rate (HR): CO = SV x HR. Both stroke volume and heart rate are affected by age, cardiac structure and cardiac function (3), and stroke volume is also dependent on preload, afterload and contractility.

Blood pressure (BP) measures afterload and has an influence on CO and peripheral vessel resistance (PVR) BP = PVR x CO (4). When the PVR is high, the heart will have to produce more energy to overcome this resistance; this will be at the expense of the expelled volume from the left ventricle (1). As previously stated the flow (CO) of the pulmonary circulation is equal to the systemic circulation, although the PVR in the latter is 15 times larger, due to different histological structure in the arteriolar vessels (1). During exercise there is a reduction of PVR due to vasodilatation, so exercise gives an increase of systolic BP due to an increase of CO. Diastolic BP only increases slightly due to vasodilatation (VD). A lack of increase or a decrease of systolic BP during exercise is a strong indication of severe cardiac dysfunction. (PVR will decrease due to normal VD, but the heart is not able to increase the CO, resulting in a decrease of BP) (4).

The maximal oxygen uptake (VO\(_{2}\text{max}\)) is considered to be one of the most useful variables to predict the outcome, and evaluate the severity of heart failure, or to appraise the efficacy of
therapy (5, 6). Since cardiac output can be measured with spirometry in a specific setup, this might be a more reliable parameter of cardiac function.

CO (Q) can be considered as the quotient of VO\textsubscript{2} and oxygen extraction. During exercise, CO increases in an almost linear manner, concomitant with increasing oxygen consumption (VO\textsubscript{2}) (4). Peripheral tissue oxygen extraction causes a difference in oxygen concentration between arterial and venous blood. If the volume that circulates through the peripheral tissues and the amount of oxygen extraction is known, it is possible to calculate VO\textsubscript{2}. This is also known as the Fick principle, as demonstrated in the following formula:

\[ \text{VO}_2 = Q \times (\text{CaO}_2 - \text{CvO}_2) \]

Oxygen extraction depends on aerobic enzyme activity, mitochondrial density, hemoglobin concentration, and capillary density in the exercising musculature (3). The arterial-mixed venous O\textsubscript{2}-difference increases during exercise, due to the lower O\textsubscript{2}-concentration in the mixed venous blood (6). It is important to keep in mind that there are several restrictions associated with the use of VO\textsubscript{2}. There is a big individual difference due to biology, heredity, body size, and illness (6). Hence, it is more interesting to standardize for weight when comparing VO\textsubscript{2}. However, it is important to realize that VO\textsubscript{2max} does not increase proportionally to the gain of body weight in fast growing children (6). VO\textsubscript{2max} is the uptake of oxygen during maximal exercise at MSL (mean sea level) (6). It is considered the golden standard to evaluate fitness and general state of cardiac health and can be determined at maximal effort using spiroergometry (6, 8).

While exercising, muscles will be forced to work harder, and intracellular processes will generate more energy. During sustained exercise of moderate intensity, the generation of energy occurs exclusively by aerobic oxidation of carbohydrates and fatty acids. When the intensity of exercise is increased, the demand of the muscles for O\textsubscript{2} will be raised (6). Accordingly, VO\textsubscript{2} increases linearly with exercise intensity (8) (Figure 1). A higher oxygen uptake will be achieved by intensifying the function of several cardiorespiratory mechanisms up to a point at which no further increase in VO\textsubscript{2} occurs, despite increasing exercise intensity. At this point the maximal oxygen uptake is reached (7). Within a ‘healthy’ individual VO\textsubscript{2max} will be limited by the maximal capacity of the cardiorespiratory system to capture oxygen from the inspired air, the binding rate of

![Figure 1](image-url)
hemoglobin, and the efficiency of transport by the blood (9). Stroke volume, as part of cardiac output, can be seen as the most important factor to increase oxygen uptake during exercise (6). Moreover, according to the formula above, we can conclude that cardiac output increases linearly with VO₂ (7, 8). Bongers et al. state that for each L min⁻¹ increase in VO₂, CO increases in children as well as in adults with approximately 5 L min⁻¹ (8). If VO₂ fails to increase at a rate appropriate to the demand of the intracellular processes which generate energy for the muscles, as seen in diseases of the cardiovascular system, the onset of anaerobic oxidation of carbohydrates will occur earlier, resulting in lactic acidosis at a lower work rate (7).

For many years now, the maximum oxygen consumption (VO₂max) has been considered as a ‘powerful independent prognostic index’ and gold standard for physical performance evaluation, and thus also for cardiac function (10). It has been demonstrated that it is a valuable prognostic marker in patients with heart failure, and has been used to determine the timing of heart transplantation (5, 10, 11). However, VO₂max is influenced by factors such as muscle deconditioning, motivation, obesity, age and gender (10, 11). Chomsky et al. state that VO₂max has a prognostic value, but cannot be disconnected from its correlation with CO (11). This is why we want to investigate if CO, as a pure hemodynamic test, is more valuable in evaluating congenital heart defects than VO₂max. It is commonly known that CO at peak exercise is a strong prognostic factor for heart failure, and an important tool in diagnosis and follow up of efficacy of therapy in heart diseases (5). CO might be a more specific parameter with greater value as a predictor of exercise capacity and with prognostic value for congenital heart defects (5).

In this study, we want to compare noninvasively measured CO and other parameters in children born with different congenital heart defects (CHD). It is our goal to evaluate the prognostic value of CO measurement during cardiopulmonary exercise test (CPET) and the validity of this test during follow up of children with CHD by comparing it to the gold standard VO₂max. Evidently, there is a difference in caliber of CO between adults and children, as well in a resting state as during exercise, though this difference depends more on the change in SV rather than the change in HR (4). As previously stated (cfr. 1 Cardiac Output definition and value), CO will increase during exercise due to a raised SV and HF. The rise in SV is possible because there is a greater venous return of the blood to the heart during exercise (6). Nonetheless, the value of SV will attain a plateau when 50% of VO₂max is
reached, and declines again at an exercise level close to the limit of tolerance (12). Then, cardiac output will further increase due to the still increasing heart rate, in a linear way with the gradually incrementing load (6). Heart rate will be higher in young children, as a compensation for their smaller heart and lower SV, resulting in a higher maximal HR (4). Children are less capable of increasing SV compared to adults. Ergo, during submaximal steady state exercise, HR and total PVR are greater at a given workload, whereas SV and CO are lower compared with adults (4).

In pediatric patients we commonly use Indexed Cardiac Output (CI) to express the heart flow. This is CO with a correction for body surface area, this is necessary because there is a large individual variability in growth stage between young children, and this index enables better comparisons of data (1). The mean normal value of CO at rest for average sized adults without cardiac pathology is approximately 5 to 6 L/min (7). In the pediatric age group, the mean CI value for normal controls is 3 L/min/m² (13). When there is no intra-cardiac shunt, it is safe to assume that the blood flow from left and right heart is the same (1, 13).

2. USE OF SPIOERGOMETRY IN CPET: DEFINITION AND METHOD
Cardiopulmonary exercise testing (CPET) is a clinical method where the patient is observed and monitored while he is performing a physical exercise conform an imposed protocol. During the test, several objective parameters are collected (14). These parameters are the response of the cardiovascular and ventilator system to a, preferably maximal, exercise test (7). Maximal effort is desirable to obtain reliable and reproducible data when cardiac output is investigated. Dynamic exercise is preferable above static exercise because it can evoke maximal or near-maximal cardiovascular responses (4).

CPET allows us to evaluate exercise capacity, cardiovascular responses, ventilatory responses and gas exchange capacity by means of the determination of several parameters e.g. blood pressure and heart rate, VO₂max and O₂pulse, maximal work rate, anaerobic threshold, tidal volume and minute ventilation (6, 8). It is important to realize that the cardiovascular measurements are interrelated with the gas exchange measurements and will require spirometry to be performed (7). Other often used equipment are ECG, blood pressure measurement (RR), metabolic cart and pulse oximeter (14).
2.1 Ergometry

Different types of exercise tests have been investigated in the past. Nowadays, the treadmill and cycle ergometer are the most common modalities used for this purpose (6, 8, 14-16). Concerning CHD analysis, the cycle ergometer will be the preferred modality, taken into account that the child is tall enough to reach the pedals (6, 8). Because the upper body is more stable on a cycle ergometer, it makes it easier to obtain reliable ECG and blood pressure measurements (8, 14). The cycle ergometer makes it possible to set the resistances very precisely through workload, as a result the workload is not estimated from the patient’s weight (as is the case with the treadmill), but is determined very accurately from the established resistances (8, 14). However, premature test termination due to non-cardiorespiratory cause is more likely on a cycle ergometer because children will stop more often as a result of fatigued legs (6, 8). VO2max yielded by cycle ergometer will typically be 5 to 10% lower than treadmill testing, due to less muscle groups that are involved, and not due to fatigued legs (6, 8, 14).

To acquire a maximal performance from the test subjects, the workload is increased according to a set way. Workload is expressed in Watt or Watt/kg. The maximal workload (Wmax) can be predicted based on body weight or height (6). Wasserman et al. formulated following equations for Wmax, valid for children below the age of 12 years old (7):

Boys: Wmax = 4.36 x height – 4.55 or Wmax = 0.053 x weight – 0.30
Girls: Wmax = 2.25 x height – 1.84 or Wmax = 0.029 x weight – 0.29

There is no single standard testing protocol for children because of the wide variety of ages and testing indications. Basically there are two general protocols, an incremental protocol or a continuous ramp protocol. Increments in workload can be increased by 5, 10, 15 or 20 Watts per unit of time, where in continuous ramp protocols the workload will increase in a linear way. The ramp protocols provide better hemodynamic and gas exchange responses during exercise than the incremental protocols (14). Incremental protocols can be more difficult for children as for each step-up they are likely to terminate the test due to muscle fatigue.

2.2 Spirometry

Peak exercise capacity is defined as "the maximum ability of the cardiovascular system to deliver oxygen to exercising skeletal muscle and of the exercising muscle to extract oxygen from the blood" (15). Consequently, exercise tolerance is determined by three factors: pulmonary gas exchange, cardiovascular performance, and skeletal muscle metabolism (15). When an exercise test shows reduced exercise capacity, one of these systems will be limited.
It is important to realize that heart diseases might result in abnormal breathing and gas exchange responses to exercise, as do many disorders of the lungs. Similarly, pulmonary diseases can primarily cause abnormalities in cardiovascular responses to exercise (7).

As cited before (*cfr. I Cardiac Output definition and value*) $\text{VO}_{2\text{max}}$ is a very useful variable to evaluate exercise capacity. Carbon dioxide production in one minute ($\text{VCO}_2$) and minute ventilation ($V_E$) reflect correctly on cardiovascular health and fitness. The minute ventilation ($V_E$) is the gas volume that is ventilated per minute by the lungs. $V_E$ is the sum of the minute alveolar ventilation ($V_A$) and the minute dead space ventilation ($V_D$) (7). The volume of air that is ventilated by the alveoli in the lungs ($V_A$) depends on the $\text{pCO}_2$ of the pulmonary arterial blood. The relationship between these parameters can be expressed in the following formula: $\text{VCO}_2 = V_A \times \text{PaCO}_2/\text{PB}$. $\text{PaCO}_2$ is the arterial, or ideal, alveolar $\text{CO}_2$ tension and $\text{PB}$ is the barometric pressure (7). $V_{E\text{max}}$ is the value of $V_E$ at maximal exercise intensity, or at $\text{VO}_{2\text{max}}$.

Measurement of $\text{VO}_2$, $\text{VCO}_2$ and minute ventilation is yielded by spirometry, using a calibrated respiratory gas analysis system (8). During spirometry, the subject breathes through a facemask with a flow sensor that receives continuous air flow (15). When breathing through a mask, extra dead space is added to the respiratory system. Gas analysis should reflect the dead space of the patient and not that of the equipment. It is preferred that equipment dead space doesn’t transcend 59 mL in children with BSA >1.0 m$^2$ and 39 mL in children with BSA < 1.0 m$^2$ (17). The influence of dead space on cardiopulmonary gaseous exchange data in children has not been properly addressed, but may significantly confound the interpretation of data (17). The expired gas is analyzed breath by breath. Next, volume and fractional concentrations of oxygen and carbon dioxide in the inspired and expired gas phases are determined breath-by-breath using rapidly responding gas analyzers (4, 6). The breath-by-breath measurement of $\text{O}_2$-uptake is methodologically complex but will produce the most accurate data (17). At the end of the expiration $\text{O}_2$- and $\text{CO}_2$-pressure are measured in the expired air: $\text{PETO}_2$ and $\text{PETCO}_2$ (6). These values approximate the concentration of the gases in the blood best, because the contamination with air from the pulmonary dead space is minimal at the end of the expiration (6). Determination of the $\text{CO}_2$-concentration in the bag makes it even possible to determine CO in a noninvasive manner (*cfr. 3.2.2 Indirect Fick*).
It is possible to predict VO$_{2\text{max}}$ while using a ramp-protocol for exercise testing in children. It is interesting to compare the measured VO$_{2\text{max}}$ with the calculated VO$_{2\text{predicted}}$ to check if the test person lives up to the expectations and give a direct view on divergent test results (17). Wasserman et al. defined following calculations, respectively for boys and girls (7):

Boys: predicted VO$_{2\text{max}}$ (mL/min) = 52.8 x body weight – 303  
Girls: predicted VO$_{2\text{max}}$ (mL/min) = 28.5 x body weight + 288

Consequently, it is possible to estimate the maximal load W$_{\text{max}}$ from the predicted VO$_{2\text{rest}}$ and VO$_{2\text{max}}$ (6).

Predicted W$_{\text{max}}$ = (predicted VO$_{2\text{max}}$ – predicted VO$_{2\text{rest}}$) / 10.3

With VO$_{2\text{rest}}$ = (Height (cm) x 2) – 100

The oxygen uptake efficiency slope (OUES) is a parameter that is useful as an alternative for VO$_{2\text{peak}}$ (16). OUES describes the relation between the VO$_2$ and the logarithm of V$_E$ throughout the entire exercise test. Since OUES can be seen as the slope of the logV$_E$/VO$_2$ curve, it is safe to assume that OUES does not significantly alter with increasing exercise intensity once a particular level of exercise intensity is reached. Then, maximal exercise is not required while determining OUES. In contrary to VO$_{2\text{max}}$, OUES can predict cardiopulmonary fitness even if the effort is submaximal. OUES can be seen as a reflection of the efficiency at which oxygen is extracted by the lungs and used in the periphery (16).

Other parameters as respiratory gas exchange ratio (RER) and the ventilator equivalents for O$_2$ and CO$_2$ can be calculated (4, 6). RER is the quotient of VCO$_2$ and VO$_2$. It is a parameter that is indicative for the amount of CO$_2$ that is exhaled per minute compared to the amount of O$_2$ that is inhaled (6, 18). However RER is determined at the level of the lungs, it is considered as a reflection of the metabolism of the muscles (6). When the muscles can only use fat as energy source, aerobic oxidation would entirely be sufficient as energy delivering system. Hence, the amount of O$_2$ consumed will be high compared with the amount of CO$_2$ that is produced. In this condition RER will be 0.7 (6). When carbohydrates are used as energy source, energy can be yielded by aerobic and anaerobic oxidation. As anaerobic oxidation is more important, the ratio VCO$_2$/VO$_2$ will be higher. RER will be 1.0 when muscle’s energy is exclusively delivered by oxidation of carbohydrates (6). Thus, RER is also useful as parameter to indicate the transition from aerobic to anaerobic metabolism (18). At rest RER is usually around 0.7-0.8.
When RER is determined at rest before exercise testing, it is possible that the test subjects have a RER of approximately 1.0 because they start to hyperventilate due to the face mask or mouthpiece, although this isn’t always the case (6). As soon as the test starts, RER will initially decrease, but will increase when exercise testing is maintained for a longer period and could be above 1.0 at the end of the test. Values as high as 1.10 have been reported for children without pathology (17, 18). This can be explained by the hyperventilation of the subject, which is a compensation mechanism for the higher CO₂ production due to a greater anaerobic contribution (6). During cycle ergometry testing, values of RER at VO₂max are usually higher than values yielded by treadmill running (17). RER has more value as an individual parameter to determine whether VT is reached in each subject, but it doesn’t allow us to compare patient populations concerning exercise capacity. The reason for this is due to the small variability in value, RER will not deliver statistically significant differences between populations, and RER increases less in children compared to adults.

The ventilator equivalents VE/VO₂ and VE/VCO₂ are calculated parameters that describe the VE necessary to respectively take up 1 liter of oxygen, and breath out 1 liter of carbon dioxide (6). The lower the equivalent value, the lower the ventilation volume needed to take up or to breath out O₂ or CO₂, and the lower the work of breathing (6). During exercise, VE will be driven on by effort and muscle fatigue, which is more pronounced in cycle ergometry. In patients with cyanotic CHD, VE will increase not only because of anaerobiosis but also because of cyanosis. This will make interpretation of VE values more difficult (17). VE/VO₂ and VE/VCO₂ can be presented in a curve where the slope can be used to determine the anaerobic threshold (AT) (Figure 2)(6, 8).

Oxygen pulse (O₂pulse) is a parameter for the oxygen consumption per heartbeat (6, 18). When it is safe to assume that oxygen extraction is not decreased, oxygen pulse can be
considered as an indirect measurement of the stroke volume according to the derived formula $O_2/HR = SV \times (CaO_2 – CvO_2)$ (6, 18). In children oxygen pulse increases with age, this is compatible with the regularity that SV increases during the development of the child (6). Wasserman et al. stated that it is pathological when the oxygen curve reaches a plateau during a maximal exercise test (6). This could be indicative for a pulmonary vascular disease (6). Another situation where this parameter would be useful is the assessment of whether exercise capacity is limited by impaired blood flow. Reduced blood flow per heartbeat causes a lower oxygen delivery in the exercising muscles, resulting in a lower overall oxygen consumption by the muscles whereby the oxygen consumption per heartbeat (e.g. O$_2$pulse) will be decreased (17).

2.3 Maximal Effort

The ideal exercise test accedes following criteria: it has a duration of approximately 8 to 12 minutes for adults and adolescents and 6 to 10 minutes for children; it is easy to perform; maximal load is reached; the test gives a good view of exercise capacity; and it can be performed by all children (8).

When performing an exercise test, it is preferred that maximal effort is reached. Maximal effort requires that the subject continues exercise until VO$_{2\text{max}}$ is reached. Oxygen consumption increases rapidly when dynamic exercise begins. While VO$_2$ is in steady state, other parameters as heart rate, CO, blood pressure and pulmonary ventilation are maintained at reasonably constant levels. In adults, VO$_2$ reaches a plateau at VO$_{2\text{max}}$ and no further rise is observed with further increases in the rate of work (4). Moreover, VO$_{2\text{max}}$ could decline slightly (cfr. 1 Cardiac Output definition and value). It can be a real challenge to reach a VO$_{2\text{max}}$ state in individuals who are not motivated or unable to perform at maximal effort. To avoid this, the VO$_{2\text{max}}$ is interchangeable with VO$_2$ at peak exercise (VO$_{2\text{peak}}$), this is the highest possible attainable exercise level in the patient (8, 16). Children increase their oxygen consumption approximately 10-fold during exercise; adults normally obtain a 10- to 15-fold increase. Oxygen consumption is strongly related to fat-free body mass, and when VO$_2$ is indexed by body weight and gender, the difference in oxygen consumption between genders become minimal (4).

As mentioned before, many times during an exercise test a plateau in VO$_2$ is not reached, therefore to determine VO$_{2\text{peak}}$, subjective criteria are used. These include signs of intense
effort, such as sweating, facial flushing and unwillingness to continue. Objective criteria are HR > 180 or RER > 0.99 (8, 18).

The HR response during exercise testing is relatively easy to measure, what makes it the most common analyzed variable in pediatric and adult exercise physiology (8). Since CO increases linearly with VO$_2$, it is safe to assume that HR increases almost linearly with VO$_2$ and, by extension, exercise intensity (7, 8). At approximately 75% of VO$_2$peak, HR will start to level off when VO$_2$peak is further approached (8). The change in HR is modulated by the cardiac autonomic nervous activity and the sinus nodal function (6, 8). The increase during exercise depends on age and gender. In trained subjects, the HR response will be delayed, where in deconditioned subjects there will be a much faster increase in HR. Although it is generally known that HR$_{peak}$ will decline proportional to the increase in age, this decline will only start from the age of 16 (18). Therefore it is more useful to evaluate the recovery of the HR after the exercise, which will be much slower in subjects with lower endurance capacity. When evaluating heart rate during exercise, it is important to realize that determinants such as type of exercise, body position during testing, and environmental conditions affect the HR regulation (8). The values of HR$_{peak}$ achieved on a cycle ergometer will be 5 to 10 % lower than these yielded by CPET on a treadmill (6, 8). Children without heart disease usually have a HR$_{peak}$ between 183 and 210 bpm and do not show a decrease or increase during childhood, pediatric heart patients have a HR$_{peak}$ that is 10 to 15 bpm lower, partly due to chronotropic incompetence (6).

Another useful parameter to determine if the patient is close to VO$_2$peak is the ventilatory threshold (V$_T$) or anaerobic threshold. This is the point where the oxygen supply is no longer sufficient for the oxygen demands of the muscles; it is the moment where anaerobic metabolism will complement the aerobic metabolism to produce the necessary amount of energy the muscles require. V$_T$ is usually expressed as a percentage of VO$_2$ above which transition to anaerobic metabolism commences (6, 18). In the blood, the increasing contribution from anaerobic metabolism is reflected by a rapidly rising lactate level (17). Graphically, a sudden increase in the slope of CO$_2$ production can be seen during exercise when transition takes place and V$_T$ has been reached (18).

V$_T$ can be determined both by invasive and noninvasive methods (6). The invasive method whereby a blood sample is taken and the lactate level is determined, is very convenient in exercise testing. Although Davis et al. stated that the validity of the use of a reference value as
anaerobic threshold is doubtful, generally a blood lactate level of 4 mmol/L is considered as the threshold value (6). On the other hand, other authors suggest that 2.5 mmol/L would be a more appropriate threshold value in 11- to 16-year olds (17). In younger children fixed reference values for lactate are not even a suitable criterion for submaximal performance (17).

The noninvasive method to determine $V_T$ involves detection of alterations in gaseous exchange parameters (17). As stated before (cfr. 2.2 Spirometry), the graphical presentation of the ventilatory equivalents $V_E/VCO_2$ and $V_E/VO_2$, where they are presented as a function of the testing time, can give a decisive answer about the point of turnover (6, 8, 17) (Figure 2). This method is known as the ‘equivalent method’. When an abrupt increase in the $V_E/VO_2$ appears while the $V_E/VCO_2$ remains equal, it is safe to conclude that the ventilatory threshold has been reached. The end-tidal values for $O_2$ and $CO_2$ ($PETO_2$ and $PETCO_2$) are behaving in a comparative way as the respective ventilator equivalents, and are also conclusive for the attainment of the $V_T$ (17). An alternative noninvasive method is known as the V-slope method (8, 17). This method involves the detection of the point at which the linear slope of the relation between the $VCO_2$ and $VO_2$ changed. This point can be seen as the $V_T$ (8). In healthy children the $V_T$ will take place between 40-70% of $VO_2peak$, where Reybrouck et al. found that the $V_T$ will always be below normal value in children with CHD, whatever the type of heart defect they might have (18), (19).

3. DIFFERENT CARDIAC OUTPUT MEASUREMENT METHODS

An ideal method would be noninvasive, reproducible, easy to perform and cheap, but up until recently only invasive ways of determining the CO were known. (20). The advantages of invasive techniques, such as accuracy, are often outweighed by the inherent risks of invasive catheterization (e.g. subclavian artery puncture, cardiac arrhythmias, bleeding, embolism, clotting and infection) (21).

3.1 INVASIVE

3.1.1 Pulse Dye Densitometry

This invasive method allows intermittent determination of CO. It is based on the theory that the output of the heart is the same as the amount of dye rapidly injected, divided by its average concentration in the arterial blood after a single circulation through the heart. The technique uses a polyethylene catheter introduced 20 cm into the antecubital vein, used for
dye injection, and another catheter inserted 15 cm into the brachial artery, for dilution measurement, by the Seldinger technique (12, 22). Subsequently an amount of 10 – 15 mg of Cardio-Green (indocyanin green), which had its absorption maximum in the infrared part of the spectrum (at 805 microns), is rapidly flushed into the circulation (22, 23). Then, using a densitometer, the density of the dye is measured (24). The cardiogreen method probably gives the most accurate measurements of CO during exercise (23). The CO is calculated from the obtained dye dilution curve according to the Stewart Hamilton Principle (25). This states that the CO is equal to the volume of dye that is injected, divided by the area under the dilution curve, measured downstream. To collect accurate measurements, appropriate signal detection is mandatory. Hence, poor peripheral circulation or vasoconstriction can endanger correct data collection (24). A downside of this method, other than its invasiveness, is that it doesn’t allow measurement of ‘beat to beat’ changes, and it requires CO that is stable for more or less than 10 seconds during exercise and 30 seconds at rest (23).

3.1.2 Transpulmonary Thermodilution
As a variation of the pulse dye densitometry method, a thermodilution method is used. The main differences with the dye dilution method are that only one catheter is required and that the indicator is a small amount of cold saline. The temperature is measured downstream from the injection site, if the cold saline causes a small change in the blood temperature, the CO is higher than in a situation where a large temperature decrease occurs. We have to acknowledge that there is, as with any technique, an existing potential for errors. There can be a false measurement when there is recirculation or disappearance of the indicator, when the flow into the sampling catheter changes or when the CO changes. Therefore it is important to collect the data during steady-state conditions. This technique is limited in clinical practice because of its invasiveness, cost and complexity (20). Another problem is the lack of agreement with the direct Fick calculations (20). Data have shown that aside from the invasiveness, thermodilution is not a desired technique for CO determination during exercise due to overestimation of CO at low values and underestimation of CO at high values (20).

3.1.3 Right Heart Catheterization
This technique is executed to determine the heart function and to measure the pressures in the heart and lungs. Using a pulmonary artery catheter, observations are made of the blood flow through the heart and the pressures inside the heart and lungs are measured. Many risks come with this procedure, such as excessive bleeding, pneumothorax, arrhythmias, infection, air
embolism etc. This way of CO measurement is not favorable for small children, especially not during exercise.

3.1.4 \( \text{O}_2 \) rebreathing with Direct Fick

The Direct Fick principle (efr. 1 Cardiac Output definition and value) has been considered as golden standard to determine CO for many years. Blood flow to an organ or through the body can be calculated using a marker substance if the amount of the substance that is added to, or removed from the blood and the concentration of the marker substance in both arterial and venous blood are known. The direct Fick principle is using oxygen as marker substance (Fick oxygen technique):

\[
\text{CO} = \frac{\text{VO}_2}{(\text{CaO}_2 - \text{CvO}_2)} \quad (7).
\]

Where \( \text{VO}_2 \) is \( \text{O}_2 \)-consumption (mL/min); \( \text{CaO}_2 \) is oxygen content in the systemic arterial (outgoing) blood circulation and \( \text{CvO}_2 \) is oxygen content in the systemic venous (incoming) blood circulation. They respectively correspond to the oxygen content in the venae and arteiae pulmonales, assuming that there is no intra cardiac shunt. Oxygen content will be high in the pulmonary venous vessels that transport blood that underwent gas exchange in the lungs, because they transport blood outgoing from the heart that is delivered to the tissues where metabolic gas exchange will happen. On the other hand, oxygen content will be lower in the arteiae pulmonales and systemic venous vessels, since muscle oxygen extraction has happened.

Both \( \text{CaO}_2 \) and \( \text{CvO}_2 \) can be estimated using following formula:

\[
\text{O}_2\text{-content} = (\text{Hb} \times 1.34 \times \text{O}_2\text{SatFraction}) + (\text{PO}_2 \times 0.0031) \text{ while Hb in g/dL, O}_2\text{SatFraction in % saturated blood and 1.34 represents the volume O}_2 \text{ (mL) per g Hb.}
\]

Blood collection and capillary saturation measurement are necessary to measure hemoglobin content and arterial oxygen saturation. Because venous oxygen saturation cannot be measured in capillary blood, the assumption is made that when arterial oxygen saturation is normal (99%), venous oxygen saturation will approximately be 75%. It is possible to estimate \( \text{CvO}_2 \), but the exact value cannot be derived from this formula. Exact determination of \( \text{CvO}_2 \) requires a sample of the pulmonary artery blood, which makes cardiac catheterization necessary (26).
The use of the direct Fick technique is limited by its invasive nature. It makes measurement of CO unlikely in exercise testing that does not take place in a heart catheterization lab. Furthermore, direct Fick must suffice some other requirements to be successful. At first, while blood is collected, it is necessary that the marker substance (O\(_2\)) is thoroughly mixed with the blood; otherwise, the O\(_2\)-content will not be representative for the entire cross-section of blood. Second, respiratory rate and pulmonary volume have to be constant and last, errors in sampling and oxygen analysis must be avoided.

### 3.2 Noninvasive

#### 3.2.1 CO\(_2\)-rebreathing with Indirect Fick

The general principle of a noninvasive cardiac output monitoring system is based on the partial carbon dioxide rebreathing method. The partial \(\text{CO}_2\) rebreathing technique employs an adapted form of the Fick equation (cfr: 3.1.4 \(\text{O}_2\) rebreathing with Direct Fick) to determinate CO, using \(\text{CO}_2\) as marker substance: \(\text{CO} = \frac{\text{VCO}_2}{(\text{CvCO}_2 - \text{CaCO}_2)}\) (7, 26, 27). According to previous equation, CO stands for pulmonary blood flow, generally expressed in L/min (7, 26, 27). \(\text{VCO}_2\) is the volume of carbon dioxide produced by metabolic processes in the body and excreted by the lungs. (7, 26, 27). \(\text{CvCO}_2\) and \(\text{CaCO}_2\) respectively stand for estimated \(\text{CO}_2\) concentration in mixed venous and arterial blood (7, 26, 27).

The \(\text{CO}_2\) rebreathing technique is much more useful in a clinical setting compared with the direct Fick method because it does not require invasive measurements (27). Noninvasive measurement of blood gases imply indirect measurements, thus the adapted form of the Fick equation using \(\text{CO}_2\) as marker substance can be considered as an indirect Fick method (27).

Another important difference between the direct and the indirect Fick method is the rate of variability. The indirect Fick method shows an increased variability because it is using \(\text{CO}_2\) as marker substance, that has a much lower diffusion capacity as compared to \(\text{O}_2\).

The variables from the indirect Fick equation are yielded by measurements of expired gases in a rebreathing bag containing \(\text{CO}_2\) and \(\text{O}_2\). The point of measurement can be set before or during the rebreathing maneuver. Arterial and mixed venous \(\text{CO}_2\) contents are obtained from measurements of end-tidal \(\text{CO}_2\) partial pressure (Pet\(\text{CO}_2\)) made at the mouth during normal breathing and rebreathing maneuvers (27).

\(\text{VCO}_2\) can be calculated when the expired volume in one minute and the difference between expired and inspired \(\text{CO}_2\) concentration are measured.
CaCO₂ is calculated from the partial pressure of CO₂ in the arterial blood (PaCO₂) using the CO₂ association/dissociation curve. PaCO₂ can be measured during breathing - at a certain level of exercise intensity - while the CO₂ level is observed and a steady end-tidal point in the rebreathing bag is reached. The air at the end of a tidal breath has been shown to best approximate the blood PCO₂ as it is leaving the lung. Consequently, PaCO₂ can be calculated from PetCO₂ according to the CO₂ association/dissociation curve: PaCO₂ = 5.5 + (0.9 PetCO₂) – 0.0021 VT. VT is tidal volume. It is calculated by the formula PetCO₂ = FetCO₂ (BP – 47); BP = breathing frequency (28).

Correspondingly, CvCO₂ is calculated from the partial pressure of the venous blood (PvCO₂). The rebreathing maneuver brings the partial pressure of CO₂ (PCO₂) in the lung-bag system into equilibration with the PvCO₂, this is the venous blood that is arriving in the lungs. When using the equilibrium method the subject will rebreathe from a bag containing a CO₂ concentration higher than the CO₂ concentration in the mixed venous blood, causing a concentration gradient for CO₂ between the lungs and the pulmonary blood that is reverse to the natural concentration gradient. Hence, the movement of CO₂ into the lungs will be strongly limited and equilibrium will be achieved: the PvCO₂ will be equal to the PCO₂ in the lung-bag system. After equilibrium is reached, the PCO₂ of the venous blood can be measured in the lung-bag-mixed venous system to predict the mixed venous partial pressure. PvCO₂ can be determined by two techniques. This equilibrium method was first described by Collier, the exponential method by Defares (7).

The gas mixture in the bag will automatically be prepared with predefined CO₂ and O₂ concentrations, based on the VO₂ and the PetCO₂ (28). The CO₂ concentration in the bag will be at least 8.5%, and will rise to 11.5% at maximal exercise (28).

The exponential method involves the use of initially lower concentrations of CO₂: 2% at rest and 4% during exercise (28). Unlike the equilibration method, an equilibrium will not be achieved using the exponential method because the blood circulation will undo the changes due to CO₂-exchange that take place with each breath, within 12-15 seconds of rebreathing. Because equilibrium will never be reached, the fraction of CO₂ (FeqCO₂) at equilibrium cannot be measured. FeqCO₂ will be predicted from the asymptotic increase in the FetCO₂ during rebreathing. FetCO₂ can be calculated according to following formula: FetCO₂ = FasymCO₂ (1-exp^k(t+c)). FvCO₂ can be estimated from FeqCO₂. Eventually, PvCO₂ is calculated: PvCO₂ = FvCO₂(Pbar – 47) (28). The usefulness of this method is limited in
different ways: variations in pulmonary blood flow and blood circulation during the re-breathing, and pulmonary shunting will cause a significant bias of the derived value.

While performing the re-breathing maneuver, some conditions have to be fulfilled beside the CO₂ content in the bag. At first, a uniform breathing pattern regulated by a metronome is required for the exponential method. In the literature, ideal respiratory rates of 30 and 40 breaths per minute are described since rapid breathing reduces the time required for equilibrium (28). Furthermore, the bag should contain the appropriate volume of gas: 1.5 to 2 times the subject’s tidal volume is considered as most likely. In this way, complete emptying of the bag during re-breathing is avoided; this would be uncomfortable and would abort the test too quickly.

The greatest benefit of the partial CO₂ re-breathing or indirect Fick method is its non-invasive nature. Furthermore, the measurement is easily repeatable and only one analyzer is required to determine all three terms of the Fick principle.

*Validity and reproducibility of the indirect CO₂ re-breathing techniques*

Vanhees et al. studied the reproducibility of the CO₂ re-breathing techniques at rest and during submaximal exercise (28). The subjects were twelve healthy men who had to perform five graded exercise tests on a cycle ergometer, with a 1-week interval between tests. For every subject, the exponential as the equilibrium method was used two times to determine CO. Vanhees et al. didn’t notice significant differences for estimated cardiac output between duplicate measurements at rest nor at any level of exercise with both methods (28). Both methods were equally reproducible at rest, but a smaller variability was seen with the equilibrium method. Furthermore, values of CO at rest yielded by the exponential method tend to be overestimated, while the equilibrium rather underestimates CO at rest. The equilibrium method is therefore considered as more valid at rest compared with the exponential method (28). During different levels of submaximal exercise both methods produced data that were equally reproducible. The reproducibility of both methods was lowest at rest and improved during exercise and thus higher workload (28). Previous studies relate this effect to errors in the estimate of arteriovenous difference for CO₂ content at rest, caused by small random errors in the estimation of CaCO₂ and CvCO₂. At peak exercise, the data for CO were again equally reproducible (28). Exercise duration is shorter with both methods compared with the pretest where the exercise protocol was the same but no rebreathing
maneuver was executed. Moreover, exercise duration was shorter with the equilibrium method – because of the higher CO₂ concentrations at the start - compared with the exponential method. These two considerations can be explained by the appearance of unpleasant side effects when breathing for a significant time through the one-way valve of the rebreathing mask (28).

### 3.2.2 Inert Gas Rebreathing

The inert gas rebreathing technique (IGR) is based on the Fick principle and was introduced in 1912 by August Krogh (2). The method was recently improved with the introduction of the Innocor System, a small portable device that is based on a photo-magnetoacoustic gas analyzer (20). It also contains a three-way automatic respiratory valve, a facemask and a rebreathing bag. The technique is based on 15 - 30 seconds breathing from a closed system (the rebreathing bag) that contains a low concentration mixture of both an inert and a blood-soluble gas (29). The inspired gas contains 90% room air, the remaining 10% is composed of oxygen with 5% nitrous oxide (N₂O) and 1% sulfur hexafluoride (SF₆), which results in a inspired concentration of 0.5% N₂O as the soluble gas and 0.1% SF₆ as the nonsoluble part of the mixture (29). The determination of the CO is derived from a perfusion-limited change in the alveolar concentration of the soluble inert gas breathed from the closed system with known volume. When the inert gas mixture is inspired, N₂O disappears from the alveoli at an absorption rate proportional to the pulmonary blood flow (PBF) and is dissolved in blood and tissues. In the absence of shunts, PBF is held as the equivalent to CO (29). After a few breaths, the insoluble gas, SF₆, has reached an equilibrium (20). Since SF₆ stays unaltered by the PBF, and the volume and content of the rebreathing bag is known, the total systemic volume can be determined from the dilution of the insoluble gas (20). The volume of the rebreathing bag is calculated by the IGR system as a function of the patient’s height and age (20).

In order to collect accurate measurements of CO with this technique, it is very important that the rebreathing maneuver is executed correctly. This means that the participant has to be able to take steady and equally deep breaths, ideally at a rate of 20 breaths a minute, since irregular breathing interferes with reproducible measurements. These requirements can make it difficult for young children or seriously ill patients to perform the maneuver (29). IGR is only performed at rest, mild and submaximal exercise because at peak exercise, there is an irregular breathing pattern which in turn yields unreliable measurements (29).
The IGR method is a safe and noninvasive technique for easy CO determination with good reproducibility (20). Due to the ability to evaluate the degree of gas mixing and to estimate intra pulmonary shunt, the technique has the potential to measure CO more precisely than CO₂-rebreathing (2).

### 3.2.3 Doppler Echocardiography

Doppler Echocardiography is a noninvasive method to measure SV during exercise. In 1987, J. Christie et al. investigated the validity of this method, comparing it to thermodilution and Fick oximetry. They concluded that there was an agreement at each exercise stage, although Doppler echocardiography had a broader range (30). The Doppler ultrasound transducer is positioned in the suprasternal notch and measures the flow in the ascending aorta. Assuming the ultrasound beam is parallel to the blood flow and the cross sectional area of the aorta is known, the SV can be calculated at rest as well as during exercise (30). The advantage of placing the transducer at the suprasternal notch is less impediment of rigorous breathing. Other influences on the accuracy of the received signals are a tortuous aorta, chronic pulmonary disease, obesity and small intercostal spaces (30).

### 3.2.4 Electrical Impedance Cardiography

This is a relatively cheap method and can be used during steady state as well as progressive exercise. It is based on the electric conductivity of tissues (20). The following formula is used: $Z = \frac{V}{I}$, where $Z$ is Impedance (referring to alternating current), $V$ is voltage and $I$ is current. A constant sinusoidal current is sent through the thorax, $V$ is measured and $Z$ is calculated, to eventually obtain SV. The most practical placement of electrodes can be seen in Figure 3. The impedance used in this method consists of 3 components. $Z_0$ is the baseline thoracic impedance, it reflects the electrical characteristics of the fluids, mass and air in the thorax. $Z_H$ reflects the respiratory activity and $Z_R$ depends on the cardiac cycle. While $Z_0$ doesn’t change in time, $Z_H$ and $Z_R$ determine the thoracic impedance changes.

In the study of H.J. Bogaard et al. they found a correlation coefficient of 0.90 between SV measured by electrical impedance cardiography and thermodilution (31). However, arrhythmias, intrathoracic water content and movement artefacts limit its accuracy.
3.2.5 Other methods
Throughout the years there have been many attempts to find the ideal method to determine the CO as accurately as possible. Among these techniques are radionuclide ventriculography, radionuclide angiography or pulse waveform analysis (10, 20). For several reasons, these procedures of obtaining CO are not suitable for use during heavy exercise loads, and all these manners require a set time to be performed. This implicates that simultaneous peak CO can not be determined (10). Cardiac magnetic resonance is a very precise method to determine CO, but this has proved to be too expensive, and time consuming. Also, it is difficult while exercising, and often requires sedation in young children, making this an unpractical method for regular follow up (20).

METHODS

1. LITERATURE RESEARCH
For a better understanding of the subject and to generate a deeper and more complete research paper, we looked for more information in scientific literature. To obtain these scientific articles, we used the search engine ‘PubMed’, and narrowed down our search results by using the following ‘Mesh’ terms in different combinations; ‘Partial CO\(_2\) Rebreathing’, ‘Congenital heart defect’, ‘Cardiopulmonary test’, ‘Exercise test’, ‘Spirometry’ and ‘Cardiac output’. We also used the user manual of the Oxycon Pro to guide us and provide us with the necessary information. In total we found 53 articles that gave us a better insight in the topics discussed in our introduction, we sited 38 of these articles. After having read all these articles, we can conclude that the majority of the studies agree that CPET is a very useful and valuable way for follow up of exercise performance and cardiac function in children with CHD (and in general people with heart disease). There has been a lot of research for finding a useful, cheap, repeatable and noninvasive way to measure CO, but there hasn’t been a break through to a general consensus concerning the best method in pediatric patients, although the CO\(_2\)- rebreathing method stood out as one of the most reproducible and stable ways to evaluate CO in children. Literature says that inert gas rebreathing is the most accurate noninvasive way to determine CO during CPET, but this is only the case when the patient is capable of taking even, steady breaths, which isn’t obvious in young children. Most studies have been conducted with small patient populations, used adults, or studied few different CHD over a
short amount of time; these are all factors that could origin bias. We could only find 9 articles who described studies with a specific pediatric population.

We found 24 recently published articles about the CO$_2$-rebreathing method used for an assessment of patients with cardiopulmonary related diseases. All studies agree that the CO$_2$-rebreathing method has the tendency to underestimate the CO at a resting state (28, 32, 33), but has fairly accurate measurements during exercise. The CO$_2$-rebreathing method has been compared to the Inert Gas Rebreathing (IGR) method, thermodilution, Doppler, pulmonary artery catheterization and many others, and most studies agree that accurate values are obtained when compared with other CO-measurement techniques, thus making the CO$_2$-rebreathing method interchangeably with these other methods. Although, more research is necessary to determine which method is acceptable in which patient population, and which method isn’t (2). Some studies suggest that the CO$_2$-rebreathing method could be useful during thoracic surgery or to detect low CO after cardiac surgery, especially when the risk-benefit ratio does not favor using pulmonary artery catheterization (32, 34). As stated before (cfr. CO$_2$-rebreathing with Indirect Fick), there are two different methods for CO$_2$-rebreathing, e.g. the exponential and the equilibrium method. There is no difference in repeatability between both methods, although, at rest there is a smaller variability with the equilibrium method. Vanhees et al. stated that the equilibrium method caused more unpleasant side effects in the subjects, such as headache, dizziness, and extreme dyspnea, causing the subjects to terminate the test prematurely; these side effects can be credited to the higher CO$_2$ concentration in the rebreathing bag, and are less seen when using the exponential method (28).

2. Study Upset

In our study, we used the CO$_2$-rebreathing method by indirect Fick with the equilibrium method, this has the tendency to underestimate CO at rest, but during exercise, this method produces valid estimates of CO (28). For good execution of this method, cooperation of the subject is required and technically demanding equipment is necessary (cfr. 2.3 Equipment). Since the introduction of computerized analysis, much observer bias could be eliminated, resulting in a higher reproducibility. It is also recommended to let the subject practice the maneuver a couple times before the CPET is started.
For this study, CO was measured before and after the test (circa 15 seconds after reaching maximal intensity), for it is very difficult to estimate 90% of the maximal intensity level of the subject and it isn’t evident to make a young child breath in a high CO$_2$-containing bag for 30 seconds. The Oxycon Pro Jaeger machine automatically calculates the gas mixture in the bag. The company doesn’t release the formula for this calculation. The CPET protocol that was used for this study is an incremental ramp protocol on a cycle ergometer. The predicted Watt for increasing the load was calculated by using the child’s weight/4. This is the amount of watts the load will increase by minute.

2.1 Study Subjects

The subjects that were recruited for this study were children that had performed a cardiopulmonary exercise test between 2006 and 2012 at the Department of Pediatric and Adult Congenital Cardiology of the University Hospital of Ghent. In this time interval 855 cardiopulmonary exercise tests were carried out for various reasons: as a follow-up in case of congenital heart defects or after a surgical cardiac intervention, or only because of concerns about inexplicable chest pain, fatigue, suspicion of rhythm problems or other exercise induced complaints. When the same child performed multiple exercise tests, we only included the data of their last test. Patients with indistinct heart anomalies who didn’t fit into either of our ‘congenital heart defect’-group (Tetralogy of Fallot, Coarctatio, Transposition of the great arteries or Univentricular hearts) were also excluded from our study. Patients with normal structured hearts were excluded when they showed for example Kawasaki, or drug induced heart muscle diseases or when their data were incomplete. Eventually we selected 60 patients as control group, and 134 patients with congenital heart defects, ages 5-19 years (Figure 4, Figure 5).
The patients were matched as nearly as possible for age, height and weight as well as sex ratio with the control group. While matching our groups for age, weight and height, we came across the finding that age did not have a normal distribution within the control group (Figure 6). This can be explained by the fact that CPET is often performed right before puberty (9-10 years old), this might be explained by the fact that this is the age when kids join sport teams for the first time, and thus, start exercising on a regular basis.
2.2 Data Collection

Patient data were retrospectively obtained from scanned files in the electronic patient dossier (EPD) like patient letters and reports of the exercise tests. When data were absent or incomplete, it was revised in paper patient documents and in the software program of the Oxycon Pro Jaeger measuring system. The ethical committee of the University Hospital of Ghent approved the study.

At the Department of Pediatric and Adult Congenital Cardiology, it is standard to use the European Pediatric Cardiac Code from the Association for European Pediatric and Congenital Cardiology (AEPC). Our patients were assigned a code according to their spectrum of detected heart defects (35). The patients were classified based on the most important pathology. The most prevalent pathologies were Tetralogy of Fallot (TOF), transposition of the great arteries (TGA), coarctatio aortae and univentricular hearts. Patients without congenital heart defect were classified as ‘normal structured heart’. Furthermore, children that had undergone a liver transplantation formed a separate entity; these patients were tested for another study, and could be an interesting addition to this study.

Patients with normal structured heart, who were referred for atypical chest pain or palpitations, but whose file was cleared negative for heart disease, were used as control population. This group consists of 30 boys and 30 girls from whom all considered parameters were available.

2.3 Equipment

Cardiopulmonary exercise test was performed on an electronically braked cycle ergometer (Ergoselect 100K, Ergoline, Germany) with a ramp protocol. All subjects were encouraged to exercise until exhaustion or notice of any adverse event. A 12-lead electrocardiogram (ECG, Marquette, GE Healthcare, UK) and pulse oximetry (Radical, Masimo Corp., US or Accutor Plus, Masimo Corp., Irvine, CA, USA) were recorded continuously throughout the test. Cuff blood pressure was measured every 3 min (Tango, SunTech Medical, Morrisville, NC, USA). A breath-by-breath gas-exchange analysis was made using a calibrated expiratory gas analysis system (Oxycon Pro, Jaeger, Viasys Healthcare GmbH, Höchberg, Germany). With this method, the highest 30-s average of oxygen consumption during the last phase of exercise was defined as the peak VO₂. Predicted values were obtained from established values from age- and sex-matched controls, and measured values were expressed as the percentage of the predicted peak VO₂ (7). A % predicted peak VO₂ < 82% was identified as subnormal exercise capacity. Minute ventilation (VÉ, y-axis) and carbon dioxide production (VCO₂, x-axis) were
plotted in a curve and its slope $V_E/VCO_2$ was defined as an index of gas-exchange efficiency during exercise. (HR) variation was obtained from ECG recordings.

3. Statistical Analysis

IBM SPSS Statistics 22 was used. We used the Shapiro-Wilk test of Normality to explore the normal distribution of parameters within the control group and the study subjects. Data are reported as mean value ± standard deviation (SD). The relation between CO and other parameters was assessed by Pearson or Spearman rank correlation analysis. To explore difference between the descriptives (age, weight and height) we used the Unpaired Student’s T-test (when there was a normal distribution) or the Mann-Whitney U test (when there wasn’t a normal distribution). We used the Wilcoxon signed rank test, to explore if VO$_{2\max}$ and VO$_{2\text{predicted}}$ had similar values within the control population. For all comparisons, p<0.05 was considered statistically significant.

We computed a new variable, “CO$_{\text{rest}}$”, by using ‘Compute Variable’ and multiplying CI$_{\text{rest}}$ with body surface area (BSA). We calculated the oxygen pulse, ”O$_2$ pulse”, by dividing VO$_{2\max}$ with HR$_{\max}$. To compare the difference of rest values and values at maximal exercise intensity we computed the variables ‘Difference between rest and max’ for SV, SVI, CI and CO, by subtracting the max values with the rest values.
RESULTS

Patient and control demographics are summarized in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Controls (n=60)</th>
<th>Univentricular (n=19)</th>
<th>Coarctatio (n=30)</th>
<th>TOF (n=58)</th>
<th>TGA (n=27)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n (SD)</td>
<td>n (SD)</td>
<td>n (SD)</td>
<td>n (SD)</td>
<td>n (SD)</td>
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<tr>
<td>Age</td>
<td>11.47 (2.3)</td>
<td>11.74 (3.2)</td>
<td>13.20 (2.9)</td>
<td>12.02 (2.7)</td>
<td>12.04 (3.2)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>151.18 (13.6)</td>
<td>148.59 (17.6)</td>
<td>154.23 (13.1)</td>
<td>150.72 (16.6)</td>
<td>153.52 (17.1)</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>43.56 (12.4)</td>
<td>38.94 (12.6)</td>
<td>45.83 (11.7)</td>
<td>42.83 (16.9)</td>
<td>44.83 (14.4)</td>
</tr>
<tr>
<td>BSA (m²)</td>
<td>1.34 (0.2)</td>
<td>1.25 (0.3)</td>
<td>1.39 (0.2)</td>
<td>1.32 (0.3)</td>
<td>1.37 (0.3)</td>
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<tr>
<td>Sex (M:F)</td>
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<td>1.7:1</td>
<td>1:1:1</td>
<td>2:1:1</td>
<td>2:1</td>
</tr>
</tbody>
</table>

TOF Tetralogy of Fallot, TGA Transposition of the Great Arteries, BSA Body Surface Area

Frequency tables were used to find outliers and exclude them. Exclusion criteria were measurement errors while data were collected. Subjects with abnormal values for SV_max, CI_max or SVI_max were completely excluded from the analysis, where subjects with exclusively abnormal SV_rest, CI_rest or SVI_rest values were only rejected from the dataset for these abnormal parameters. CO measurement at rest and at maximal exercise are two separate measurements. As in the literature we also reported more measurement failures at rest (n=23). We excluded 6 patients with abnormal high CO values at maximal exercise according to values from the study of Myers et al. (36).

1. ASSESSMENT OF RELIABILITY OF CO MEASUREMENT

We wanted to see if there is a significant correlation between CO and VO₂max (gold standard) within the control group. There wasn’t a normal distribution (p<0.001) for CO, this means we used the Spearman correlation test. To check if our control population’s test results corresponded to the expected values, we tested the difference between their VO₂max (x=1743.87(518.5)) and VO₂predicted (x=1765.45(597.7)). The null hypothesis, which states that both values were equal, could not be rejected (p>0.05), which indicates acceptable test results for the control population.

There was a positive statistically significant (p<0.001) correlation coefficient between CO_max and VO₂max (0.678), CO_max and Load (0.647), CO_max and V_E (0.615), CO_max and O₂pulse (0.721), CO_max and SV_max (0.568) and CO_max and test duration (0.714). There was a positive statistically significant (p=0.001) correlation coefficient between CO_max and RER (0.428). There was no statistically significant correlation between CO_max and HR_max (p=0.199), and
CO\textsubscript{max} and V\textsubscript{E}/V\textsubscript{CO\textsubscript{2}slope} (p=0.199). We also found a strong statistically significant (p<0.001) correlation between CO\textsubscript{max} and Weight (0.680). Mean and standard deviation are shown in Table 2.

<table>
<thead>
<tr>
<th>Controls n=57 n(SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age</strong> (years)</td>
</tr>
<tr>
<td><strong>Weight</strong> (kg)</td>
</tr>
<tr>
<td><strong>Height</strong> (cm)</td>
</tr>
<tr>
<td><strong>CO\textsubscript{max}</strong> (L/min)</td>
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<tr>
<td><strong>HR\textsubscript{max}</strong> (bpm)</td>
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<tr>
<td><strong>V\textsubscript{Emax}</strong> (L/min)</td>
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<tr>
<td><strong>\textit{O}_2\textit{pulse}</strong> (mL/beat)</td>
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<tr>
<td><strong>\textit{VO}_2\textsubscript{max}</strong> (mL/min)</td>
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<td><strong>\textit{VO}_2\textsubscript{max}</strong> (mL/kg/min)</td>
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<tr>
<td><strong>Load\textsubscript{predicted}</strong> (W)</td>
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<tr>
<td><strong>RER</strong></td>
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<tr>
<td><strong>SV\textsubscript{max}</strong> (mL)</td>
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<td><strong>Test Duration</strong> (min)</td>
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</table>

2. **Comparison of characteristics between Congenital Heart Defects and control group**

After assessing the reliability of CO measurement within the control group, we wanted to see how this parameter behaved when we compared the results of our heart patients with the control group. The group of congenital heart defects was split and each group (Coarctatio, Univentricular hearts, Tetralogy of Fallot and TGA) was compared with the control group. Each individual in the CHD groups was matched with an individual in the control group, for sex, age, weight and height.
2.1 Coarctatio

After matching for age (12.62±2.4 years), height (152.46±13.1 cm) and weight (43.89±11.2 kg) and attaining same sex ratio’s (1:1), we found no statistically significant differences between the control group and coarctatio for HRmax (p=0.282), VO2max (p=0.859), O2pulse (p=0.466), V̇E/VCO2slope (p=0.963), Load (p=0.996), V̇Emax (p=0.861), and RER (p=0.087). Only ‘Test Duration’ had a statistically significant (p=0.020) difference (Table 3a).

Since there were many missing values and outliers for SVrest, SVmax, SVDiff, COrest, COmax and CODiff, we had to rematch for these values to be able to compare with the controls again. We became a new mean±SD for age (12.60±2.7 years), height (150.40±12.8 cm) and weight (44.66±13.0 kg). The coarctatio population became much smaller (n=20) but remained large enough to obtain valid comparisons. There was no statistically significant difference between the coarctatio and control population for SVrest (p=0.972), SVmax (p=0.906), SVDiff (p=0.724), COrest (p=0.775) and CODiff (p=0.743). There was a statistically significant difference in for COmax (p=0.036) (Table 3 (a&b).

### Table 3 (a&b)

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<tr>
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<td>152.85 (12.7)</td>
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<td>Weight (kg)</td>
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<td>Test Duration(min)</td>
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</tr>
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<td>Load (W)</td>
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<td>1.00 (0.1)</td>
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</table>

HR Heart Rate, CO Cardiac Output, RER Respiratory Exchange Ratio, VE Minute Ventilation

<table>
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<td>SVmax (mL/beat)</td>
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<td>SVDiff (mL/beat)</td>
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SV Stroke Volume, CO Cardiac Output
2.2 UNIVENTRICULAR

After matching for age (11.74±3.3 years), height (148.59±17.6 cm) and weight (38.94±12.6 kg) and attaining same sex ratio’s (2:1), we found a strong statistically significant difference (p<0.001) for HR_{max}, VO_{2max}, and V_{E}/VCO_{2}slope. For O_{2} pulse, the difference was statistically insignificant (p=0.094). For ‘Test Duration’ and Load we found a statistically significant (p=0.004) difference, for V_{Emax} we found a borderline statistically significant (p=0.041) difference. RER (p=0.103) did not have a statistically significant difference (Table 4 (a&b)).

Due to several missing values and outliers for SV_{rest}, SV_{max}, SV_{Diff}, CO_{rest}, CO_{max} and CO_{Diff}, we were obliged to rematch to be able to compare the parameters between the control population and the univentricular heart patients. We obtained a group of 12 patients without outliers and missing values. A new mean±SD for age (12.00±3.2 years), height (148.56±15.4 cm) and weight (40.24±12.9 kg) were retrieved. There was a statistically significant difference for SV_{rest} (p=0.023), SV_{max} (p=0.003), CO_{rest} and CO_{max} (p<0.001). We did not find a statistically significant difference for SV_{Diff} (p=0.498) and CO_{Diff} (p=0.526) (Table 4 (a&b)).

Table 4 (a&b)

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</tbody>
</table>

HR Heart Rate, CO Cardiac Output, RER Respiratory Exchange Rate, VE Minute Ventilation
2.3 Tetralogy of Fallot

After attaining same sex ratio’s (1.6:1) and matching for age (11.51±2.5 years), height (146.86±14.7 cm) and weight (39.15±14.1 kg), we found a statistically significant difference between the control group and the Tetralogy of Fallot population for HRmax (p=0.004), VO2max (p=0.006), O2pulse (p=0.038), Test Duration (p=0.006) and Load (p=0.013). There was no statistically significant difference for RER (p=0.199), VE/VCO2slope (p=0.130), and VEmax (p=0.171) (Table 5 (a&b)).

Because of missing values and outliers for SVrest, SVmax, SVDiff, COrest, COMax and CODiff, a new matching was required to be able to compare these parameters between the two groups. We obtained a group of 37 patients without outliers and missing values. A new mean±SD for age (12.35±2.6 years), height (153.80±17.8 cm) and weight (44.28±17.0 kg) were retrieved. There was a statistically significant difference for SVmax (p=0.007) and COrest (p=0.015). We did not find a statistically significant difference for SVrest (p=0.095), COMax (p=0.789) SVDiff (p=0.411) and CODiff (p=0.803) (Table 5 (a&b)).

Table 5 (a&b)

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<th>Controls n=28 (SD)</th>
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</tbody>
</table>

**TOF** Tetralogy of Fallot, **HR** Heart Rate, **CO** Cardiac Output, **RER** Respiratory Exchange Ratio, **VE** Minute Ventilation
2.4 Transposition of the Great Arteries

After attaining same sex ratio’s (2:1) and matching for age (12.04±3.2 years), height (153.52±17.1 cm) and weight (44.83±14.4 kg), we found a statistically significant difference for HR_{max} (p=0.002), and ‘Test Duration’ (p=0.015). There was no statistically significant difference for VO_{2max} (p=0.163), O_{2}pulse (p=0.723), Load (p=0.604), V_{E}/VCO_{2}slope (p=0.815), RER (p=0.322), and V_{E}max (p=0.762) (Table 6 (a&b)).

Due to missing values and outliers for SV_{rest}, SV_{max}, SV_{Diff}, CO_{rest}, CO_{max} and CO_{Diff}, we were obliged to rematch to be able to compare the parameters between the control population and the TGA group. We obtained a group of 17 patients without outliers and missing values. A new mean±SD for age (12.32±2.6 years), height (156.11±15.1 cm) and weight (47.40±14.5 kg) were retrieved. There was no statistically significant difference for SV_{rest} (p=0.678), SV_{max} (p=0.279), SV_{Diff} (p=0.426), CO_{rest} (p=0.498), CO_{max} (p=0.069) and CO_{Diff} (p=0.576) (Table 6 (a&b)).

Table 6 (a&b)

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<td>2:1</td>
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</tbody>
</table>

|                  | TGA n=19 | Controls n=38 |
| Age (years)      | 12.32(2.6) | 11.83(2.2)    |
| Weight (kg)      | 47.40(14.5) | 45.08(13.5)   |
| Height (cm)      | 156.11(15.1) | 153.55(14.5)  |
| SV_{rest} (mL/beat) | 73.05(53.9) | 63.58(29.3)   |
| SV_{max} (mL/beat) | 109.47(29.6) | 100.76(29.0)  |
| SV_{diff} (mL/beat) | 37.18(27.3) | 37.18(27.3)   |
| CO_{rest} (L/min) | 5.89(3.5)   | 5.88(2.4)     |
| CO_{max} (L/min) | 14.69(5.3)  | 12.70(5.3)    |
| CO_{diff} (L/min) | 4.98(4.1)   | 5.13(3.7)     |
| RER              | 1.02(0.1)   | 1.00(0.1)     |
| Sex Ratio (M:F)  | 2.8:1       | 2.8:1         |

TGA Transposition of the Great Arteries, HR Heart Rate, CO Cardiac Output, RER Respiratory Exchange Ratio, VE Minute Ventilation
DISCUSSION

Cardiopulmonary exercise testing is one of the most sensitive tests to evaluate causes of exercise intolerance, since exercise amplifies the abnormalities of all organs that couple external to cellular respiration (7). There is no test that will be capable of quantifying improvement or worsening of function of these organs better than CPET, making it a very effective tool to identify patients with heart disease, grade their functional capacity objectively and predict their cardiac reserve. It is also an excellent way to distinguish primary cardiac from ventilatory-based exertional dyspnea (37). It is very important to evaluate each child during rest as well as during exercise in a controlled environment, to be able to make adjusted recommendations for each child individually about what intensity level the child can handle. It can also be a reassurance for anxious parents (17). The peak oxygen consumption (VO₂max) has traditionally been considered the gold standard for physical performance. It has been demonstrated that it is a valuable prognostic marker in patients with heart failure. However, VO₂max is influenced by factors such as muscle deconditioning, pulmonary diseases and gas transportation by the blood. This is why we wanted to investigate if noninvasive CO measurement, as a more cardiac specific parameter, has a more complete prognostic value for children with congenital heart defects than VO₂max. We put 180 children with CHD through CPET and measured CO using the CO₂-rebreathing method, as well as other parameters. Over the course of 3 years we also collected data of a large group (n=265) of normally structured heart patients who underwent CPET because of benign chest pains. To determine the clinical utility of CO measurement at maximal exercise, we investigated the whole group CHD (n=134). In 97 subjects CO measurement at maximal exercise was possible. Of the 37 subjects to whom CO measurement failed, 9 measurements failed due to technical defects e.g. problems with the module, depletion of the gas tank etc., so not because of patient-related factors. This was seen in our dataset as a long gap: a longer period of time where more consecutive measurements failed. The remaining 28 failed measurements could be due to patient-related factors, however it is possible that some of these failed measurements were also due to technical defects. When we don’t take the absent measurements, due to technical defects, into account, we can assume that 97 of the 125 performed measurements were successful, what matches a percentage of 77.6%. Possibly, the success ratio is higher, but we did not interpret failed measurements as technical defaults when they were not part of a long gap in the data. It seems plausible to us that the success rate of CO measurements at maximal
exercise was higher in the group without CHD. Because of this, it is safe to assume that the CO₂ rebreathing technique is reasonably easy to perform in children.

To make sure that this group of children with no underlying CHD, who came to the hospital for CPET, are a representative sample of the population of healthy children with normal structured hearts, we tested the difference between their VO₂max and VO₂predicted. There was no statistically significant difference between these two parameters, which indicates that our 60 selected controls form a representative sample of the population.

1. CORRELATION OF CO IN THE CONTROL GROUP

We started our study by exploring if CO was indeed a valuable parameter for the assessment of fitness and cardiac function. We did this through analysis of the correlation between COmax and the classic CPET-spirometry parameters within the control group. Our data for VO₂max (46.02±9.2 mL/kg/min for the boys and 36.66±7.9 mL/kg/min for the girls) are conform previous findings of Bongers et al. The strong positive correlation between COmax and VO₂max in the control group showed us that these parameters could be equally useful for the interpretation of fitness. This agrees with the Fick principle, when you assume no confinement exists for the oxygen extraction at the tissue level, COmax will have a positive correlation with VO₂max. Takken et al. established that the primary influence on CO is SV, and we can assume that this will be within normal range for our control group with normal structured hearts (6). Since COmax is a more direct hemodynamic parameter (cfr. 1.Cardiac output definition and value) it can give a broader comprehension of the fitness level and prognosis of the patient.

There is a strong positive correlation between COmax and VEmax. VEmax is the gas volume that is ventilated per minute by the lungs at maximal effort. The VE is driven by exercise and muscle fatigue, which will be more pronounced in cycling (17). As said before (cfr. 2.2 Spirometry) VEmax is the sum of the minute alveolar ventilation (VA) and the minute dead space ventilation (VD) (7). This can reflect on a decreased, as well as an increased exercise capacity. When the patient has a high exercise capacity, the VEmax will be increased due to a higher VA. During exercise the gain of VA will be driven by the pCO₂ of the blood that is arriving in the lungs. Low exercise capacity, as seen in CHD patients with pulmonary atresia, can also present with a high VEmax due to ventilation-perfusion mismatch that will lead to an increased VD. The rise in VD will provoke a less efficient gas exchange, which will require a higher ventilation rate to expulse the same amount of CO₂ as normal subjects would (38). As in the literature this
will coincide with a steeper $V_E/VCO_2$ slope (39). Our finding that there was no statistically significant correlation between $CO_{max}$ and the $V_E/VCO_2$ slope in the control population, agrees with the hypothesis that a steeper slope reflects on a decreased exercise capacity. Chua et al. found that cardiac patients with a higher $V_E/VCO_2$ slope had a poorer prognosis (40). We presume, as stated before (*cfr. 2.2 Spirometry*), that $V_E/VCO_2$ is a more beneficial parameter for pulmonary evaluation, since a high $V_D$ can occur as a pure pulmonary defect, which makes $V_{Emax}$ and $V_E/VCO_2$ slope valuable parameters to assess cardiac function, but both parameters can be altered by extra-cardiac problems. Where evidently pulmonary and cardiac problems are inextricably joined in patients with CHD, it is good to be precautious about the interpretation of origin of the limited exercise capacity (e.g. pulmonary or cardiac).

There was no correlation found between $CO_{max}$ and $HR_{max}$. This can be logically explained because $HR_{max}$ is a parameter that shows a big interindidual difference while it isn’t so that greater values for $HR_{max}$ automatically indicate greater exercise capacity. For example, a younger, small child can reach a high $HR_{max}$ while its exercise capacity approximates nowhere near the exercise capacity of an older, taller child with a lower $HR_{max}$.

For the assessment of whether exercise capacity is limited by impaired blood flow we calculated the parameter $O_2$ pulse. We found a strong positive correlation with $CO_{max}$, which indicates that our control population did not have a reduced $O_2$ extraction at the cellular level or a decreased $SV$ (41). All these observation are congruent with the findings of a strong correlation between $CO_{max}$ and $SV_{max}$.

During exercise, children will in particular use fat as an energy substrate, this can only be consumed in an aerobic way, causing RER to have a lower value. RER is a useful parameter...
as a marker for effort and an indicator of the contribution on anaerobic metabolism (17). In our control population the mean value for RER was 0.99±0.08, which indicates that most of our control patients were able to reach their anaerobic threshold.

We can conclude that CO is a valuable parameter, corresponding with VO$_{2\text{max}}$ and therefore useful as a reproducible parameter for assessing fitness and cardiac function.

2. COMPARISON OF HEART PATIENTS WITH THE CONTROL GROUP

2.1 COARCTATIO

This acyanotic heart defect is relatively straightforward and usually defined as simpler CHD. It has the tendency to have less influence on the exercise capacity in surgically corrected children (42). Still, it is known that adults with surgically corrected coarctatio of the aorta have a reduced exercise tolerance than what would be expected in healthy persons, and are more prone to develop obesity due to their declined level of physical activity (43, 44). We found no difference between the patients with coarctatio and our controls except for ‘test duration’ and CO$_{\text{max}}$. The coarctatio patients weren’t able to maintain the exercise performance as long as the control population, but they attained higher values for CO at maximal intensity. The difference for CO$_{\text{max}}$ between coarctatio and the control population can be explained by large age difference - even after matching – between both populations. Consequently, the shorter test duration for the coarctatio’s cannot be explained by their cardiac impairment but is due to their lower level of fitness, that includes less developed muscle mass, decreased lung capacity and poorer blood values. There hasn’t been much research about patients with coarctatio and their VO$_2$ or CO, since essential hypertension is a more leading symptom. Although we think it would be interesting to investigate CO measurement more profoundly in this patient population.

2.2 UNIVENTRICULAR

Patients with univentricular heart defects undergo surgical correction of their CHD at infant age. Their pulmonary circulation cannot be supported by the right ventricle, since it is eluded by the Fontan procedure. Absence of the right ventricle may seriously impair the cardiopulmonary function, but there can be other reasons for the abnormal exercise response in these children (17).

We found that VO$_{2\text{max}}$ and CO$_{\text{max}}$ were both significantly lower for the univentricular hearts compared with the control population. The fact that both these parameters have a statistically
significant difference compared with the control group indicates that our previous findings concerning the correlation between VO$_{2\text{max}}$ and CO$_{\text{max}}$ were correct. Another implication of this finding is that children with univentricular hearts have a diminished exercise capacity. This is illustrated by the endowment that they score significantly lower for workload, test duration, and V$_{\text{Emax}}$. There is also a much steeper V$_{E}/VCO_2$ slope for the univentricular patients, as previously stated this is conform with a poorer prognosis. This could be due to comorbid impaired lung function or, a failing cardiovascular circulation whereby CO$_2$ arrives at a slower rate from the peripheral tissues to the pulmonary capillary bed where gas exchange takes place. We did not find a statistically significant difference for RER, but as discussed before (cfr. 2.2 Spirometry.), the individual values for RER are so small, that it is unlikely to obtain statistically significant differences between groups.

The VO$_{2\text{max}}$ and CO$_{\text{max}}$ difference with the control group is supported by the significant difference we found for SV$_{\text{max}}$. Since SV$_{\text{max}}$ is lower, we expected the O$_2$ pulse to be decreased as well. At least, this is what we could extract from the literature (17), although we couldn’t confirm this with our results.

McManus et al. state that most cyanotic CHD’s are known to have chronotropic insufficiency. We can confirm this with our results where univentricular patients have a significant lower HR$_{\text{max}}$ than the control group. This can be caused by several factors such as alterations in myocardial and sympathetic catecholamine reserves, damage to the sinus node, a decreased sensitivity of the atrial mechanoreceptors through damage to the AV-junction or an alteration in the sinus node function (17).

2.3 Tetralogy of Fallot

Tetralogy of Fallot (TOF) is a more complex CHD with decreased pulmonary blood flow and a right to left shunt due to a VSD. Apart from a subvalvular pulmonary stenosis there is often a pulmonary branch arterial stenosis present. These are not easy to assess during surgery and the residual lesions could give rise to unfavorable hemodynamics or a decreased peak oxygen uptake (17, 45).

We found significant lower values for VO$_{2\text{max}}$ compared to the control group, this is in agreement with Wessel et al. where they state that adults with repaired TOF are known to have only 80% of the exercise capacity of untrained adult individuals without congenital heart defects (46). In our study, we didn’t find a statistically significant difference for CO$_{\text{max}}$ between TOF and the control population, which is not in line with our expectations. This
could be explained by the high amount of missing values of CO-measurement in the girl-population of the TOF group, which makes the ratio boys:girls difficult to match appropriately. Maybe significant values would have been obtained if we analyzed boys and girls separately, or had a much bigger control population to start with. TOF is a cyanotic CHD that is corrected by surgical repair during the first years of life. After surgical repair TOF can no longer be considered as a cyanotic CHD, but chronotropic incompetence is still existent and contributes to the lower VO$_{2\text{max}}$ (46). This chronotropic incompetence can be seen in the significant lower value for HR$_{\text{max}}$ we found. Besides, our study revealed a lower value for SV$_{\text{max}}$, but we found a higher difference in value for HR$_{\text{max}}$ than for SV$_{\text{max}}$, which implicates that chronotropic incompetence has the most influence on the lower exercise capacity (e.g. CO$_{\text{max}}$) for TOF patients.

The significantly lower O$_2$ pulse may indicate a reduced O$_2$ extraction at the cellular level or a smaller SV. Considering our previous finding about SV$_{\text{max}}$, we could ascribe our lower O$_2$ pulse to this lower SV$_{\text{max}}$. Marcuccio et al. had the same results about the significantly lower SV$_{\text{max}}$, but they correctly raised the question regarding the role that O$_2$ extraction at the cellular level plays in exercise and what happens when the limit of O$_2$ extraction is reached (13). In our opinion it would be interesting to add these measurements during CPET for TOF patients, but this creates a pitfall, by making the measurements invasive.

RER was similar to the RER of controls, which shows us that they are capable of reaching their maximal capacity, although the load at maximum capacity was significantly lower than the control group, as well as the test duration (39). This demonstrates again that TOF patients have a lower intensity capacity.

Rhodes et al. state that there is a correlation between the degree of pulmonary atresia of the outflow tract, and the steepness of the V$_E$/VCO$_2$slope during exercise. This can be explained by the pulmonary blood volume distribution (ventilation/perfusion mismatch), and consequent SV decrease. Therefore, there will be a compromised gas exchange and thus more rapid ventilation as compensation. This is not supported by our findings, where there is no significant difference in V$_E$, and the mean V$_E$ for TOF patients is even lower than for the control group. This might be due to better surgical repair of the TOF with conservation of the effective ventilating space (V$_A$) and lower dead space (V$_D$) (39).

The two groups in our study differed significantly in CO$_{\text{rest}}$, although there was no significant difference for SV$_{\text{rest}}$. This could be due to HR$_{\text{rest}}$, however we have no data of this parameter.
in our study. Marcuccio et al., partially in contrary to our findings, didn’t find any difference for resting values for HR, SV and VO₂ (13).

2.4 Transposition of the Great Arteries

Arterial repair procedures for TGA greatly transformed the prognosis for newborn children with TGA (47). These children generally have an increased pulmonary blood flow but inadequate mixing between the pulmonary and the systemic circulation (17).

Our results show that patients with TGA, after arterial switch operation, suffer from chronotropic incompetence, this is in agreement with what we found in other articles (48). We didn’t find any other significant difference with our control group. Since the arterial repair procedure is commonly used, TGA patients have become one of the patient groups who perform best at exercise testing compared with the control population.

In general we found articles about the exercise capacity of TGA patients with atrial repair. Paul et al. found a statistically difference for HRₘₐₓ, VO₂ₘₐₓ and O₂pulse (47). The reduced HRₘₐₓ can be justified by the time-progressive loss of a “sinus” rhythm, and an increased incidence of atrial arrhythmias. Damage to the sinus node or the intra atrial conduction tracts can be caused by the surgical repair or by the incomplete rotation of the great arteries.

Overview of the range for VO₂ₘₐₓ, SVₘₐₓ and COₘₐₓ for all groups
We would also like to point out that the calculation of \( SV_{\text{Diff}} \) and \( CO_{\text{Diff}} \), which we performed because we thought that it would be interesting to determine at what degree the CHD groups were capable of increasing their stroke volume, aren’t very useful parameters. We thought we would be able to differentiate the cause of why they weren’t able to increase their CO during exercise with these parameters. When \( CO_{\text{max}} \) is low, this can be due to chronotropic incompetence (low \( HR_{\text{max}} \)) or, because of a low capacity to expand stroke volume. Children with a normal \( SV_{\text{rest}} \) and low \( SV_{\text{max}} \), have a low \( SV_{\text{Diff}} \) and thus, their low \( CO_{\text{max}} \) could be due to the fact that they aren’t able to increase their SV enough during exercise. When \( CO_{\text{Diff}} \) was low but \( SV_{\text{Diff}} \) had normal values than it should be safe to assume that chronotropic incompetence is responsible for the low \( CO_{\text{max}} \) value. Though, we weren’t able to prove this with the calculation of these parameters, which is probably due to the fact that you don’t know if the patients start with a low or normal value for SV at rest. We withhold from the literature that measurements of CO and SV at rest are less reliable (28).
3. **Study Limitations**

As a consequence of the retrospective design of this study, the studied patient cohort is subjected to bias. The presence of missing throughout the patient populations made it difficult for us to collect large enough patient groups. Although the control population was selected accurately from the population of ‘normal structured hearts’ and only children with complaints of unspecific chest pain or innocent palpitations were withheld, the control population will not be an exact reflection of the ‘healthy’ population. As $\text{VO}_2\text{max}$ and $\text{VO}_2\text{predicted}$ are equal, we assume though this a reliable control population.

In the end we realized our control population was too small, at least for the comparison with the TOF population. Maybe it would have been more adequate to match each patient with a similar control subject for age, weight and height, thus start with a larger control population. In this manner, a more reliable analysis would arise. Considering this, we realize that it would have been better to use this protocol for this study, but our study protocol was established before we realized this and it didn’t allow us to match the population in this way. Eventually, this restriction only gave rise to less accurate values in our TOF population. There was no sign of inaccurate matching for the other groups.

The patients with CHD who were referred for CPET had undergone completion of Fontan circulation or repair for TOF from a single tertiary center. This could have led to a bias with the real population of TOF patients. Within the group of CHD, it was possible to perform a cardiac output measurement at maximal exercise in approximately 80% of the study subjects. For subjects who did not undergo CO measurement at maximal exercise, $\text{CO}_{\text{max}}$ and $\text{SV}_{\text{max}}$ were reported as missing values. Naturally, these subjects were excluded for statistical analysis of these parameters. The reason why there were no data for $\text{CO}_{\text{max}}$ in these patients is caused by preterm termination of the measurements due to of unpleasant side effects that go with the CO$_2$- rebreathing method e.g. headache, dizziness and extreme shortness of breath.

In contrary to other studies, we didn’t find a statistically significant difference for some parameters in some groups of CHD when comparing with the control population. This may be due to the relatively small size of our study population in comparison with the populations that were considered in these other studies.
Lastly, CO measurement with the CO$_2$-rebreathing method requires technically demanding and expensive equipment. It is evident that not every pediatric department can afford this equipment, or especially a trained staff member to conduct the exercise tests, like we have in our hospital.

4. FUTURE PERSPECTIVES
There has already been some research about this subject, but we think it is necessary that more investigation is done. It would be interesting to set up a prospective study to be able to follow the children over many years and include more pediatric patient populations For a more complete overview of the child’s fitness capacity it would be useful to include some more parameters, e.g. heart rate at anaerobic threshold, OUES, etc. For obtaining a better and more reliable study, we think it would be wise to include many more subjects to the control population, with a more evenly spread age, weight and height difference.

Another study idea might be to compare the CO$_2$-rebreathing technique with other methods of assessing CO measurement, exercise-MRI, compared to noninvasive CO measurements.

CONCLUSION

Exact measuring of CO during exercise on a reliable and easy manner would be the Holy Grail in cardiopulmonary exercise testing. We assessed noninvasive cardiac output measurement using CO$_2$-rebreathing in a control population, whereby CO$_{max}$ correlated very well with VO$_{2max}$ and other exercise performance parameters. We withhold that CO$_2$-rebreathing method with Indirect Fick is an adequate technique to determine CO at maximal exercise in children. This is the case for children with normal structured hearts as well as for children with congenital heart defects. This method is a helpful instrument for the follow up of children with CHD, since it’s easy to perform and gives reproducible and representable data.

Further on, we investigated noninvasive CO-measurement in different patient populations with CHD, where we found that patients with UV had a significant lower SV$_{max}$ and CO$_{max}$. We withhold that CO$_{max}$ at maximal intensity reflects the severity of the CHD, therefore also the limitation of exercise capacity. Patients after coarctatio- and TGA-repair however had a normal CO$_{max}$ and SV$_{max}$. The results from this study are conform our expectations for the coarctatio, univentricular and transposition of the great arteries groups. However, the results
from the TOF group didn’t satisfy what was expected, their $SV_{\text{max}}$ was significantly smaller than the control population, and their $CO_{\text{max}}$ showed no significant difference in comparison with the control group. We expect the $CO_{\text{max}}$ to be significantly lower than the control group if our control population would have been more adequate. However, further investigation will be necessary to confirm our hypothesis.
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