Protocol Title:	Nitrate Effect on Exercise Capacitance and Hemodynamic Profile Prior to Fontan Failure
PROTOCOL NUMBER	
PHASE:	PHASE II
INVESTIGATIONAL PRODUCT:	Isosorbide dinitrate
STUDY INDICATION:	Exercise endurance and vascular function in stable Fontan patients
SPONSOR-INVESTIGATOR:	Adam Lubert, MD (CCHMC)

Good Clinical Practices

This study was conducted under Good Clinical Practices, in accordance with the Declaration of Helsinki, in compliance with the International Conference on Harmonisation (ICH) guidelines.

Nitrate Effect on Exercise Capacitance and Hemodynamic Profile Prior to Fontan Failure

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Abstract/Brief Overview:

Each year over 1,200 Fontan operations are performed on children in the United States with a congenital heart defect resulting in single ventricle physiology. This translates to over 2,500 Fontan operations annually world-wide and thousands more infants born with one functional ventricle who don't reach Fontan physiology. Fontan physiology describes a circulation where the only ventricle pumps blood to the body and blood flow to the lungs is passive, down a pressure gradient, without a pumping ventricle. Unfortunately, there can be significant long-term morbidity associated with the Fontan physiology, largely related to chronic venous congestion and difficulty traversing the pulmonary vasculature without a ventricle to power it. Nitrate based medications such as isosorbide dinitrate (ISDN) have been used in adults with heart failure demonstrating improved survival, ejection fraction, and exercise tolerance in select populations. In pediatric patients, they have recently been shown to increase venous capacitance, thereby decreasing central venous pressure within the Fontan circulation. Central venous pressure is a critical indicator of Fontan function and an instigator of morbidities in these patients. We will investigate the safety and preliminary efficacy of ISDN therapy to reduce venous congestion and improve exercise tolerance in children and adults after the Fontan operation. This will be accomplished by recruiting 15 Fontan physiology patients from the Cincinnati Children's Fontan clinic and University of Kentucky Pediatric Cardiology clinic for the investigation. We will non-invasively measure both central venous pressures at rest and during graded cardiopulmonary exercise testing. In addition we will obtain a measurement of liver stiffness before and after a 4-week regimen of ISDN therapy. Patients will be seen twice in clinic, once before and after ISDN therapy, and phone calls will be made to ensure safety, compliance, and make appropriate alterations to medications throughout the study period. Pre- and post-intervention central venous pressure and exercise tolerance will be compared.

In this investigation we hypothesize the following;

- Isosorbide dinitrate will lower central venous pressure
- The better hemodynamics associated with central vasodilation will result in greater exercise endurance and improved liver stiffness

Purpose of Study:

This study is meant to evaluate the safety and efficacy of isosorbide dinitrate in children and adults with Fontan physiology in decreasing liver stiffness and lowering exercise related venous pressure responses and increasing exercise tolerance.

Background:

Approximately 740 infants of every million live births are born with congenital heart disease complicated by single ventricle physiology; this translates to over 1,000 new patients undergoing the Fontan operation every year in the United States and likely between 50,000 to 70,000 patients worldwide

currently living with Fontan physiology who will go on to develop early heart failure(1,2). Furthermore, this number is expected to double in the next 20 years putting a huge burden on heart-failure and cardiac transplantation services(3). The current approach to managing patients born with one functional ventricle is a series of interventions that lead up to the Fontan operation(4,5). Prior to this surgery, patients died in infancy, but now this growing cohort of patients is surviving childhood only to develop heart failure, venous thromboses, venous hypertension, arrhythmias, plastic bronchitis, cirrhosis, and protein losing enteropathy. Unfortunately, patients with a failing Fontan circulation are the fastest growing cohort presenting to congenital heart programs with heart failure and the risk of heart failure 5-years after Fontan operation rises steeply, accounting for a large proportion of death in these patients(3). Though survival has improved, morbidity is also high, resulting in only a 60% employment rate and survival beyond young adulthood being a challenge(6).

Therefore, attention now must shift to the post-operative period, focusing on how to improve quality of life and prevent heart failure subsequent to the Fontan operation. While the Fontan circulation can fail for a number of reasons, venous congestion and elevated right-sided pressures (i.e., central venous pressure, pulmonary vascular resistance) contribute to some degree in nearly all cases. This congestion is primarily the result of having no sub-pulmonic ventricle and a diseased pulmonary vascular bed which are inherent to the Fontan circulation (Figure 1a)(7). Though ventricular assist devices have been utilized to support these patients, results are inconsistent and depend greatly on the cause of heart failure(8).

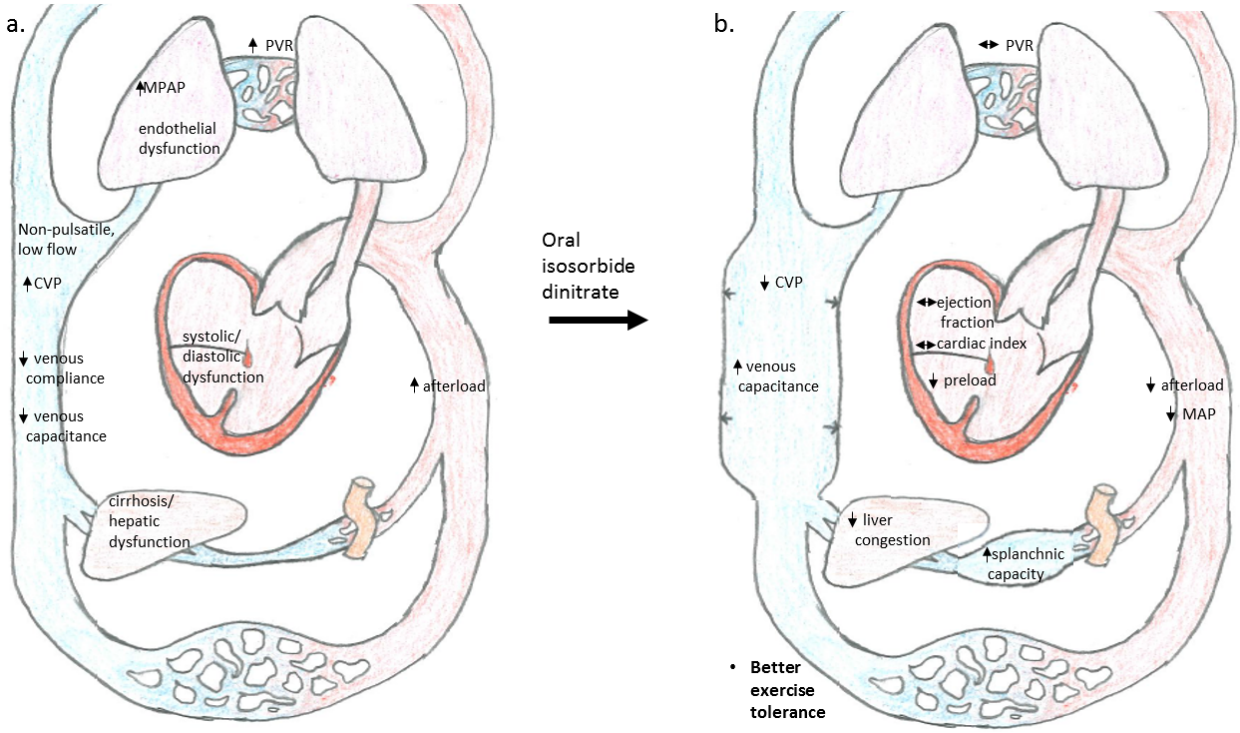

Figure 1: Primary hemodynamic effects of venous congestion manifested in Fontan circulation (a). Anticipated impact of oral isosorbide dinitrate therapy (b). CVP, central venous pressure; MAP, mean arterial pressure; MPAP, mean pulmonary arterial pressure; PVR, pulmonary venous resistance

Isosorbide dinitrate, which is metabolized to generate nitric oxide, has long been studied and used in adults with heart failure(9). It is suggested to improve symptoms by the American College of Cardiology

and the American Heart Association guidelines(9). It acts to dilate both the central venous and systemic arterial vasculature with minimal effect on pulmonary arteries(10). The V-HeFT II study demonstrated an improvement in ejection fraction and exercise tolerance in patients treated with hydralazine-ISDN. Unfortunately, many studies of ISDN include hydralazine (or other afterload reducers) in the regimen, which make the effect of the individual drugs difficult to assess. Recently, ISDN therapy immediately following Fontan operation and continued for one year after demonstrated a reduction in central venous pressure (CVP) compared to patients not receiving ISDN treatment(11). ISDN has a promising mechanism and background making it a potential medication to improve outcomes after the Fontan operation. Therefore, the purpose of this trial is to evaluate whether ISDN is safe in Fontan patients while improving exercise tolerance as well as reduced liver stiffness and venous pressure in response to exercise.

Maintaining a low central venous pressure is of critical importance in patients with Fontan physiology. This has typically been addressed by targeting the pulmonary vascular resistance; however, increasing the systemic venous capacitance (venous pool) by using venodilators has also been shown to decrease central venous pressure(12). The typically elevated central venous pressure seen in Fontan circulation is the result of decreased venous capacitance as a maladaptive response to preserve pre-load and thus cardiac output. While this is effective short-term, like many of our body's natural responses to heart failure, it is unsustainable and can be prevented with targeted therapy(13). Long-term Fontan physiology if often met with rising pulmonary and central venous pressures leading to venous congestion with detrimental impact on end-organ function, particularly the liver. Nitrate medications, such as ISDN, are effective at increasing the systemic venous capacitance, especially of the splanchnic system, and hence lowering Fontan patient's central venous pressure with the anticipated effects as depicted in Figure 1(10). At the same time, cardiac output is maintained which could lead to decreased liver congestion and improved exercise tolerance. Ultimately, the goal of this intervention is to prevent or delay long-term failure of the Fontan circulation and counteract some of the body's maladaptive responses to the Fontan circulation.

Peripheral venous pressure has been validated in Fontan physiology to correlate with central venous pressure (R=0.86, p<0.0001) through the equation CVP = 1.6 + 0.68 X PVP regardless of venous collaterals or fenestration. Mean capillary filling pressure (P_{mcf}) as another means by which to assess venous compliance of a patient. P_{mcf} correlates with the PVP after rapid occlusion of arm circulation ($P_{arm\text{-}occ}$) with a high-pressurized cuff (R=0.88, p<0.0001) through the equation P_{mcf} = 9.1 + 0.63 X $P_{arm\text{-}occ}$.(14) See figure 2. In a more compliant vasculature, P_{mcf} would be lower for a constant blood volume. All measurements of cardiac output, central venous pressure, and mean capillary filling pressure can be measured non-invasively in the clinic. Cardiac output and venous pressures will be obtained prior to starting an exercise tolerance test and will be measured and recorded at several points throughout the test, including maximal effort. Cardiac output would be calculated non-invasively by using the inert gas rebreathing (IGR) method.(15)

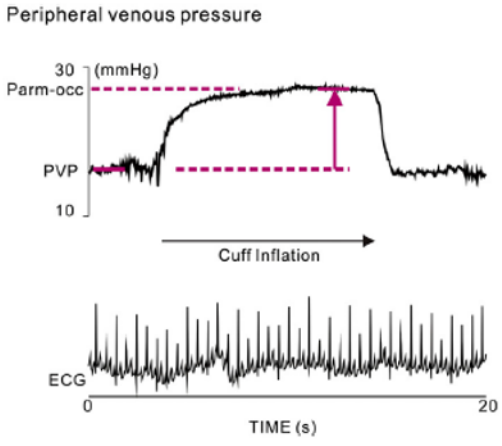

Figure 2: From Masutani et al. "Assessment of Central Venous Physiology of Fontan Circulation Using Peripheral Venous Pressure." JCTVS. April 2017

Though ISDN is generally a safe medication and is frequently used in adult heart failure patients and already being used in Fontan patients at various centers, there are several concerns which must be further evaluated in pediatric Fontan patients(16). Medication tolerance is known to occur with nitrate therapy in adults; however, this effect was not seen in children undergoing ISDN therapy for one year following the Fontan operation(11,17). Also, the impact of potentially combining ISDN with phosphodiesterase inhibitors (a common medication in Fontan patients) could increase the risk for extreme hypotension and will be a contraindication to study participation. Lastly, there is the theoretical concern that decreasing pre-load could diminish cardiac filling and thereby cardiac output, especially when exercising, but this effect is likely balanced by afterload decreasing effects of ISND acting upon other parts of the vasculature. These concerns will all be key points of the investigation into a new use of ISDN in Fontan circulations. It should be noted that our own Fontan clinic currently has multiple pediatric patients on ISDN therapy with no ill effect.

While Fontan patients are a growing population, at individual centers they still represent a very small proportion of the patients with congenital heart defects. Furthermore, they are a group of very high interest for reasons already described and existing patients are saturated with clinical trials. Therefore, obtaining large numbers of patients for long studies remains challenging in this group of patients at high risk for heart failure. Nonetheless, if shown to be an effective means of improving exercise tolerance and improving hemodynamics at a large single center Fontan clinic, this would likely warrant consideration of a randomized prospective multi-center trial which could significantly alter treatment regimens in Fontan patients.

Primary Objective

Determine the safety of a 4-week regimen of isosorbide dinitrate therapy titrated to maximal tolerated dose or 30mg and obtain estimates of the effect and variability on maximal exercise capacity in asymptomatic Fontan patients.

Secondary Objectives

1. Determine the effect of ISDN on the hemodynamic profile including non-invasively measured central venous pressure at rest and during exercise and liver stiffness.

- Central venous pressure as calculated from peripheral venous pressure at rest and exercising
- Cardiac output measured by IGR at rest and exercising
- Venous compliance
- Heart rate
- Systemic blood pressure
- Liver stiffness measured by ultrasound

2. To ensure low rate of side-effects in polypharmacy of Fontan patients

Study Design:

This will be a single arm, two-center prospective clinical trial of ISDN (up to 30 mg, 3 times daily) in pediatric and adult patients greater than 9 years old who have undergone the Fontan operation and do not have Fontan failure. The dose selected was based on current treatment protocols at the Cincinnati

Fontan clinic, literature review, and discussion with adult heart failure specialists. Fifteen patients will be enrolled from our Cincinnati Children's Fontan Clinic and collaborating pediatric cardiology clinic at the University of Kentucky. We will test the hypothesis that ISDN administration over 1-month period will be safely tolerated and improve exercise venous pressure responses, as well as exercise tolerance in Fontan patients. Our primary end point will be the change in exercise tolerance after 1 month of ISDN therapy. Secondary end points will include change in central venous pressure and cardiac output at rest and during exercise, mean capillary filling pressure, systemic blood pressure, heart rate, evidence of medication tolerance, and incidence of medication side effects including headaches.

Selection and Recruitment of Participants:

Approximately 15 evaluable subjects will be recruited from the Fontan clinic at CCHMC as well as the pediatric and adult congenital heart disease clinic at the University of Kentucky. Patients meeting eligibility criteria will be approached for participation during a regularly scheduled clinic visit at CCHMC. Alternatively, a letter of introduction approved by the IRB will be mailed to potential participants. In the letter, patients will be given the opportunity to opt-out of the study by calling the study line after receiving the letter. After one week, study personnel will call patients to further explain the study. If the patient's interest continues, study personnel will schedule a research visit for the patient at CCHMC. Written consent as outlined below will be obtained at their next clinic or study visit prior to any study procedures being conducted.

Inclusion/Exclusion Criteria

Inclusion criteria:

- Underlying Fontan physiology
- On a stable medication regimen for the past 3 months
- Nine years of age or older

Exclusion criteria:

- Pregnant or nursing
- Prior hospitalization for heart failure in past year
- Presence of uncontrolled arrhythmias within the past 6 months
- Non-cardiac conditions which significantly limited exercise
- Moderate or severe ventricular dysfunction by echocardiogram or cardiac MRI
- Currently treated with a phosphodiesterase-5 inhibitor or organic nitrates
- Concurrent enrollment in other investigational drug trial
- End stage Liver Disease (ESLD)

Study Procedures/Interventions:

Nearly all study procedures will occur at Cincinnati Children's Hospital Medical Center (CCHMC) main campus. The Heart Institute (HI) at CCHMC has an adult heart clinic where patients may be seen as well. The HI adult clinic is on site at the main hospital campus and therefore has full access to emergency medical staff onsite at the hospital.

The following procedures will occur during the study:

Baseline visit (Visit 1)

- Informed Consent
- Baseline vitals (heart rate, blood pressure, oxygen saturation, height and weight)
- Demographic, medical, and medication review
- Urine pregnancy test for all females
- Blood collection (Basic Metabolic Panel-3ml)
- Insertion of peripheral venous cannula for venous blood pressure during exercise testing and estimate of mean capillary filling pressure
- Maximal cardiopulmonary exercise testing/Ramp protocol (including spirometry)
- Liver ultrasound to assess for liver stiffness
- Medication distribution

Visit 2 (3 days [+/-1 day] after Visit 1)

- Follow-up phone call to ensure patient tolerating medication and adjust medications

Visit 3 (3 days [+/-1 day] after Visit 2)

- Follow-up phone call to ensure patient tolerating medication and adjust medications

Visit 4 (3 days [+/-1 day] after Visit 3)

- Follow-up phone call to ensure patient tolerating medication and adjust medications

Visit 5 (3 days [+/-1 day] after Visit 4)

- Follow-up phone call to ensure patient tolerating medication and adjust medications if needed

Visit 6 (4 weeks after visit 3 [+/-2 days] before discontinuing ISDN)

- Repeat vitals (heart rate, blood pressure, oxygen saturation, height and weight)
- Urine pregnancy test for all females
- Insertion of venous cannula for venous blood pressure during exercise testing and estimate of mean capillary filling pressure
- Maximal cardiopulmonary exercise testing/Ramp protocol (including spirometry)
- Liver ultrasound to assess for liver stiffness
- Recover medication vial
- Perform drug accountability
- Blood collection (Basic Metabolic Panel-3ml)

Table 1 presents schedule of procedures for this study.

Table 1: Patient Timeline

	Visit 1	**Visit 2**	**Visit 3**	**Visit 4**	**Visit 5**	**Visit 6**
	Day 0	Day 3 (+/-1 day)	Day 6 (+/-1 day)	Day 9 (+/- 1 day)	Day 12 (+/-1 day)	Day 34 (+/- 3 days
Procedures:						
Informed consent	X					
Demographics and medical history	X					
Medication review	X					
Vital signs	X					X
Urine Pregnancy Test	X					X
Venous pressure[a]	X					X
Spirometry[b]	X					X
Maximal exercise ramp test	X					X
Liver Ultrasound	X					X
Quality of Life Survey	X					X
Medication dispension	X					
Phone call to adjust medications		X	X	X	X	
Collect medication vial						X
Basic Metabolic Panel	X					X
Adverse event monitoring	X	X	X	X	X	X

[a] Venous pressure will be measured from before exercise test, every 1 minute during exercise, until 5 minutes after exercise.

[b] Spirometry will occur prior to maximal exercise testing

Pharmacologic Interventions

At the conclusion of the first visit ISDN therapy will begin at a small dose (5 mg three times daily in those targeting 20-30 mg TID, less in patients < 40 kg) and be titrated up to goal three times daily over 2 weeks (see Table 2). The medication will be continued for 4 weeks once the target dose is reached. It is an organic nitrate commonly used to prevent angina and used frequently in heart failure as well. It acts upon vascular smooth muscles to cause vasodilation of the peripheral arteries and veins. It should be taken by mouth three times per day with water. It may be taken with or without food.

Patients not tolerating the medication will be instructed to call the clinic for adjustment of medications. Severe side effects to note include pre-syncope, syncope, and severe headache. The PI will assess the patient over the phone and ask them to come in if necessary. Conversation and any changes of

medications will be documented. Less severe symptoms such as mild lightheadedness or headache should attempt to be tolerated or treated with acetaminophen prior to calling the clinic.

Isosorbide dinitrate's clinically relevant interaction is with phosphodiesterase-5 inhibitors, a common medication in Fontan patients. There is a risk for excessive hypotension and therefore, will be a contraindication to enrollment in the trial. ISDN can also affect kidney function. For this reason, a basic metabolic panel will be taken at the start of the study and 1 week after initiation of ISDN.

Side effects will attempt to be reduced by a step-wise up-titration plan, use of acetaminophen for headache, and recommendation to return to previous dose if necessary which may enhance tolerability. Furthermore, patients will be followed up with by phone call 3-5 days after any change in medication.

Diagnostic Interventions

A peripheral venous catheter (18 Gauge) will be inserted distally in an upper extremity for the measurement of central venous pressure. A blood sample will be drawn for baseline kidney function at initial visit.. Mean capillary filling pressure will be obtained by rapid occlusion of arm circulation with high-pressurized cuff as shown in Figure 2. Inert gas rebreathing apparatus will be applied for measurement of cardiac output during exercise testing.

Venous puncture

A needle will be used to draw a basic metabolic profile at Visit 1 and Visit 6 to assess renal function. The minimal amount of blood (~2 mL) will be obtained one time at each visit.

Venous Cannulation

A catheter will be placed into an antecubital vein and will be connected to a pressure monitor. Venous pressures will be monitored prior to beginning exercise and then every 1 minute during exercise and after exercise during the recovery period (5 minutes). Venous pressures measured using a peripheral venous line has previously been shown to be reflective of central venous pressure in Fontan patients. The same venous cannula will be used for obtaining mean capillary filling pressure. This same needle can be used for baseline basic metabolic profile blood draw at first visit.

Baseline Spirometry

Baseline spirometry will be performed as the best of 3 good respiratory maneuvers, directed by an experienced operator. The predicted forced expiratory volume in 1 second (FEV1) will be calculated from their height and weight and then the participant will be asked to provide three good attempts at producing their FEV1. This will be then used to calculate their maximum voluntary ventilation (MVV) with the following calculation: MVV = FEV1 x 40.

Maximal Exercise Testing/Ramp Protocol

The ParvoMedics Exercise cart will be used for cardiopulmonary exercise testing in all cases. The equipment will be calibrated and operated to the standards specified in the ATS/ACCP (2003) guidelines. Participants will initially sit at rest on the electromagnetically-braked cycle ergometer for three minutes while baseline cardiopulmonary variables are recorded (baseline phase). This will be followed by three minutes of unloaded cycling at a cadence of 60 rpm, after which the power output will be increased gradually to reach maximum exercise in 8-10 minutes. . Talking to the study subjects will be kept to a

minimum. Participants will be asked to maintain a cadence of 60 rpm throughout the test until volitional intolerance. The power output will be immediately reduced and the cardiopulmonary variables monitored for at least 2 minutes (recovery phase).

The Innocor system (Innovision, Odense, Denmark) will be used to asses noninvasive cardiac output (CO) measurements based on the Inert Gas Rebreathing (IGR) method. Innocor is in clinical use at this institution and forms part of routine clinical exercise testing. The IGR method is based on the perfusion-related change in alveolar concentration of a soluble inert gas inspired from a closed system. A second insoluble marker gas is used for the determination of lung volume. The Innocor device consists of a closed system, including a three-way automatic respiratory valve connecting a facemask; a rebreathing bag, which is filled before commencement of the rebreathing maneuver to a calibrated volume; and an infrared photoacoustic gas analyzer. The inspired gas of this system contains 90% room air, whereas the remaining 10% is composed of a mixture of oxygen with 5% nitrous oxide (N2O) and 1% sulfur hexafluoride (SF6), resulting in inspired concentrations of 0.5% N2O as soluble and 0.1% of SF6 as non-soluble component. The soluble component of the inspired gas is absorbed by the pulmonary blood flow with an absorption rate proportional to the pulmonary blood flow. In the absence of shunts, pulmonary blood flow is equivalent to CO. SF6, as non-soluble component of the inspired gas, remains unaffected by pulmonary blood flow and allows determination of lung volumes. For the measurement, patients must breathe the gas mixture from the closed system during a period of a half minute. The components of the test gas as well as the expired carbon dioxide (CO2) are continuously and permanently recorded at the mouthpiece and computed by a photoacoustic gas-analyzer.

The Innocor system will be used to assess pulmonary blood flow at rest and at peak exercise, i.e. 2 time points. Because steady breathing with constant amplitude is required to obtain reliable measurements, all participants will be familiarized with the exercise protocol and practiced the rebreathing maneuvers before the test, and we will only obtain data with the Innocor system just prior to beginning the exercise test and at peak exercise to limit any confounding of the regular exercise test data, as switching from one mouthpiece to another is required and this will minimize inaccurate data during this transition.

Liver Ultrasound

A Toshiba Aplio i800 (or equivalent) ultrasound system will be used for this study and is FDA-approved for clinical use in humans. Research imaging will include liver assessments of stiffness (ultrasound shear wave elastography), and blood flow (gray-scale and Doppler imaging). Each of these forms of imaging is FDA approved and used as part of routine care for other patients at CCHMC. While a formal imaging report will not be rendered, images will be reviewed by a study investigator or other radiologist for clinically important incidental findings. Any such abnormality would be reported to the patient's physician. All images will be stored on the Department of Radiology research PACS, with exams and images labeled using subject study ID number and date of imaging/visit. No other HIPAA-defined protected health information will be stored in the research PACS system for this investigation. Research images will be kept for the duration of the study or as long as required by CCHMC or law. All study patients should be fasting prior the ultrasound imaging being performed.

Quality of Life Survey

All subjects will complete a quality of life PROMIS © survey at both the baseline visit and final visit. Based on age, the subject will complete their designated survey. If the subject age changes from baseline to final visit, the same survey will be completed for both time points.

Obtaining Informed Consent:

Consent will be obtained before any study related procedures are performed. Our investigators and research coordinators will explain to eligible patients and their legal guardian the purpose of the study, the interventions, potential risks/benefits, and alternatives, and answer any questions. They will be informed that participation in the study is voluntary, that he/she may withdraw from the study at any time, and that this will in no way impact subsequent medical treatment or relationship with the physician. Those agreeing to participate will review and sign the IRB approved informed consent form. This form is written at a six grade level and arrangements can be made for illiterate families. The consent process will be documented in the informed consent progress note. A copy of the signed consent(s) will be kept in the patient's medical record and a copy will be provided to the patient.

Assent will be obtained in accordance with CCHMC policy for any participants 11-17.

Vulnerable Population

Children and adolescents are required for this study because the Fontan surgery is typically performed in children between the ages of 3-6. These patients will be protected from coercion and undue influence by involvement of their parent/guardian in the informed consent process and attempt to attain assent from the child for all those enrolled in the study.

Investigational Agent:

Description

Isosorbide dinitrate (ISDN) is an organic nitrate which dilates both arteries and veins and is indicated for the treatment of heart failure. Absorption of ISDN after oral dosing is nearly complete, but bioavailablity is variable but averages 25%, with extensive first-pass metabolism. Serum levels reach their maximum within an hour.

How Supplied

Isordil® (isosorbide dinitrate) Oral Titradose® Tablets will be supplied as 5-15 mg tablets by the CCHMC pharmacy according to titration.

Storage

They are round, pink tablets with one deeply scored side. They are stable until the date noted. They should be stored at controlled room temperature at 25°C (77°F), with excursions permitted to 15-30°C (59-86°F). They should be kept in a light resistant bottles.

Proposed Dose

ISDN will be administered by the CCHMC investigational pharmacy for a 2 week period of titrating up the dose followed by 4 continuous weeks of dosing of maximum tolerable dose determined by weight. Patients will be dispensed medication in 5mg, 10mg and 20 mg doses, and each patient will be given a personalized schedule for titrating up in dosage. Dosage may require halving a pill. See Table 2.

Table 2: Proposed dosing regimen (three times daily dosing)

Patient Weight	**<40 kg**	**40-49 kg**	**≥50 kg**
Visit 1, Starting Dose	0.25 mg/kg/d	5 mg	5 mg
Visit 2, Up To	0.50 mg/kg/d	10 mg	10 mg
Visit 3, Up To	0.75 mg/kg/d	15 mg	15 mg
Visit 4, Clinic Visit, Up To	1.0 mg/kg/d	20 mg	20 mg
Visit 5, Up to		x	30 mg

Patients >50 kg will have a target dose of 30 mg TID. Patients 40-49 kg will have a target dose of 20 mg TID. Patients <40 kg will have a target dose of 1 mg/kg/d. Patients >40 kg will be started on low dose ISDN therapy (5 mg TID) at first to ensure they tolerate the medication. A phone call will be made 3-5 days after initiation of therapy to inquire about symptoms, and adherence to medication. If the patient is tolerating the medication well, the dose will be increased to 10 mg TID. Once increased, another follow-up phone call 3-5 days later will be made for further inquiry about the medication tolerance and increased to 15 mg TID. After 15 mg is reached patient will be brought in to clinic to check blood pressure and draw BMP and increase the dose. In patients ≥50 kg, dose will be increased once more to 30 mg TID. Once the patient dose is stable, it will be continued for a 4 week trial. If patients are symptomatic or not tolerating the medication at follow-up phone call, attempts will be made to adjust background medications prior to lowering the ISDN dose and follow-up phone calls will be made at 3-5 days after any adjustment to therapy.

Research Data Collection and Management:

Data Collection

All data will be recorded on study-specific case report forms (CRFs). Participants will be given a study identification number that will be reported on all CRFs and source documents. Demographic data, diagnoses, prior interventions, both surgical and cardiac catheterization based intervention will be collected. Imaging reports will be reviewed for evidence of anatomic and specific hemodynamic problems of the circuit prior to recruitment of potential participants, thereby allowing us to exclude those patients. Hemodynamic characteristics and ventricular functional characteristics will be recorded at latest follow-up.

Data Management

Data management will be handled using CCHMC's standard operation procedures and guidelines. Data will be collected by designated research coordinator(s) and the physicians participating in the study. Data will be maintained in REDCap. Only study personnel will have access to data. Data will be indexed

by a study-specific subject number; the key which links this number to identifying information will be stored in a separate, secure location. All study data will be stored in a secure, confidential database.

Data Analysis:

Study Endpoints

The primary endpoint is exercise endurance as measured by VO2.

The secondary end points are the following;

- Liver stiffness
- Central venous pressure at rest and with exercise
- Peak VO_2
- Venous compliance
- Heart rate
- Systemic blood pressure

Statistical Analyses

Demographic and clinical characteristics of the patient population will be summarized using means and standard deviations or medians and interquartile ranges for continuous variables and frequencies and percents. for categorical variables.

The exercise endurance VO2 and all other study endpoints will be compared between the first and final visits.

Means and standard deviations will be calculated for exercise endurance at baseline and after one month of treatment. The 95% confidence intervals (CIs) for both the mean change and standard deviations will be calculated to obtain estimates of the population values under sampling variability. These values will provide us with a range of plausible values required to inform a rigorous sample size determination for a larger, confirmatory trial of isosorbide dinitrate on exercise capacity assuming no change under placebo. The mean change in VO2 divided the standard deviation will provide a measure of the standardized effect size. We expect a standardized mean difference of at least 0.2 standard deviation units in favor of the investigational agent would reflect a meaningful effect size to help support an encouraging (versus no effect) decision to proceed with a larger, confirmatory trial. We will also formally test whether the change in VO2 in response to isosorbide dinitrate is non-zero using a one-sample t-test or non-parametric alternative. This testing will be conducted using a one-sided alpha of 0.25 since our primary goal is to assess whether there may be preliminary evidence for efficacy and we are willing to accept greater risk of a type I error at this early stage of investigation.

Safety analyses will be conducted by reporting the counts of adverse events (AEs) and serious adverse events (SAEs). Lab values required to monitor patient safety will be determined by the investigators and DSMB. These values will be monitored and values reported to the DSMB over the course of the trial.

Estimates of preliminary efficacy and variability for secondary outcomes will be conducted using the approach described above for the primary outcome.

Sample Size Calculation

Null hypothesis testing is not directly related to achieving the proposed aims. Rather, our goal is obtain sufficiently precise estimates of the parameters needed to inform the design of a confirmatory trial. Based on our experience with this patient population we expect the mean exercise capacity VO2 at follow-up to be around 25 with a standard deviation of approximately 7. We would like to estimate the mean VO2 with a margin of error of not greater than four. A sample size of n = 15 produces a two-sided 95% confidence interval with a distance from the mean to the limits that is equal to 3.9 when the estimated standard deviation is 7. Thus, we will enroll a total of n=15 patients into this pilot study. A sample size of n = 15 produces a two-sided 95% confidence interval with a width equal to 5.9 for the population standard deviation when the sample standard deviation is 7. Thus, we expect n= 15 participants will allow us to obtain estimates of variability with sufficient precision to inform the design of a future confirmatory trial.

Risks and Benefits:

Potential Benefits:

Each patient may benefit from increasing exercise tolerance and decreasing central venous pressure for the duration of the study. If this were the case, their cardiologist would be notified at conclusion of the study to discuss longer-term nitrate therapy. In addition the information from the study may benefit Fontan patients in the future.

Potential Risks, Discomforts and Inconveniences:

Potential risks, discomforts and inconveniences of this study include three office visits, two of which will require time and for the patient to undergo intravenous blood pressure measurement. Two of the visits will also require blood draw with similar risks. IV insertion could result in minimal bleeding, bruising and pain in a small portion of people. Furthermore, they will undergo exercise testing at two of these office visits, which is associated with arrhythmias in some patients and/or discomfort.

The risk associated with the medication, isosorbide dinitrate, are primarily due to hypotension which could lead to mild headaches or potentially severe systemic hypotension. Patients will be informed of this potential complication and instructed to contact the office and seek medical attention immediately for any syncopal or pre-syncopal events. Nitrate headaches are a common effect of ISDM and can often be controlled with aspirin or acetaminophen. Such adverse events are dose-related with a low incidence and occur more commonly in the elderly. They will also be told to have the patient lie down and elevate all four extremities to increase central pooling of blood. In the event of any severe reactions, the medication would be decreased to previously tolerated dose or stopped. Furthermore, this is a primary reason for the 3-5 day follow-up phone call. In Fontan patients, lowering blood pressure could also compromise kidney function which is why a 2 week follow-up visit will be performed to assess for any

significant changes to blood pressure at the discretion of the principal investigator based on patient baseline and normal blood pressure readings.

Patients under 18 having a positive pregnancy test will have the results reported to their parents and/or legal guardian. To mitigate the emotional risk from receiving a positive pregnancy result, study staff will offer chaplain services and/or a referral to counseling services. Lastly, there is a potential loss of confidentiality by participating in the study.

Risk/Benefit Analysis

The proposed study has minimal risks associated with it, which are reasonable in relation to the knowledge that will be gained and used to create interventional programs in this high-risk population.

Assessment of Safety:

Adverse Events

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose. Adverse events will be recorded from the time of the first dose of study drug until resolution (or study completion).
Events that are unexpected and considered to be related or possibly related to the study procedures as well as breaches of confidentiality, and protocol violations must be reported to the IRB as soon as possible after discovery of the event. All adverse events will be classified by the PI for relationship (Related, Possibly Related, Unlikely Related, Not Related); severity (Mild, Moderate, or Severe); and expectedness (Expected or Unexpected). Events not meeting the criteria for prompt reporting will be reported to the IRB at the time of continuing review.

Serious Adverse Events

A Serious Adverse Event (SAE) is any undesirable occurrence associated with the use of a drug in a patient occurring at any dose, whether or not considered drug related, when the outcome is:

- Death
- Life-threatening (i.e., participant was at substantial risk of dying at the time of the AE),
- Inpatient hospitalization or prolongation of existing hospitalization,
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions or resulted in a significant, persistent or permanent change, impairment, damage or disruption in the patient's body function/structure, physical activities and/or quality of life,
- Congenital anomaly/birth defect in the offspring of a subject who received study medication, or
- Other serious (important) medical events that may jeopardize the participant and may require medical or surgical intervention to prevent one of the other outcomes are also considered to be SAEs.

Relationship

All adverse events will be classified by the PI for relationship to the study drug as: Related, Possibly Related, Unlikely Related, Not Related.

Severity

All adverse events will be classified for severity by the PI as: Mild, Moderate, or Severe.

Expectedness

All adverse events will be classified for expectedness by the Sponsor as: Expected or Unexpected. Expected events associated with the use of ISDN include:

- Headache
- Lightheadedness
- Hypotension
- Syncope
- Crescendo angina

Adverse Event Reporting:

Expedited Reporting

SAEs require expedited reporting when meeting the following criteria:

- Serious
- Unexpected
- At least possibly related to the study drug (suspected adverse reaction)

Events that are considered unexpected and related or possibly related to the conduct of the study will be reported to the IRB within seven days of discovery.
The DSMB will review SAEs within 48 hours after initial receipt of the information by the investigator(s) to review the PIs assignment of SAE as related or unrelated to treatment; to confirm the grading of toxicity, and assure that the study may continue.
Significant unplanned deviations from the protocol will also be reported as stipulated above. All other serious adverse events and non-serious adverse events will be reported at the time of submission of annual reports. Likewise, minor deviations will be reported in annual reporting.

Data Safety and Monitoring:

Data will be collected on data collection forms that will not be labeled with the patient's name, only the study number. The list of matching codes to the patient's name will be handled and retained by the study coordinator and investigator at each site. To ensure proper use and continued protection of these data, the data collection sheets will not be given to any individuals except those co-investigators and study coordinators performing data-entry.

Participant binders will be maintained with names of the subject, pertinent clinical data, and files with signed consent forms and copies of pertinent clinical and research related forms. All participant binders

and study records will be stored in locked cabinets in the PI's office or at the desk of designated research personnel. A separate Excel spreadsheet linking subject identification number to identifying information (first name, last name, medical record number, and date of birth) will also be maintained on a password-protected computer located in the PI's office or the computer of designated research personnel. Access to this information will be restricted to the PI and research staff, appropriate federal agencies, the IRB, and any sponsors of the study.

Data Safety and Monitoring Board (DSMB):

A Data Safety and Monitoring Board (DSMB) will be assembled to monitor for adverse outcomes. The DSMB will review enrollment, eligibility criteria, and safety data and will make recommendations for any alterations to the protocol or any safety concerns. The DSMB will meet after the first two patients are enrolled and then again after every 8 patients enrolled or if a serious adverse event occurs. If an SAE occurs, enrollment will be stopped until the DSMB meets to determine if the study can continue as planned or should be altered. The DSMB charter provides additional details regarding the DSMB.

Privacy and Confidentiality:

The privacy and confidentiality of patient information will be maintained in accordance with Health Insurance Portability and Accountability Act (HIPAA) regulations. All research personnel who work on this study must complete HIPPA and the Collaborative Institutional Training Initiative module on human research with direct subject interaction. No identifying data will be used in any publications that were a result from this work.

Participation Cost and Payments:

Participants from CCHMC will be given $50 per visit, plus $100 for phone calls for a total of $200 if both visits are completed, to cover the cost of time, and travel for the day. Participants will not be paid for visits not performed. Payment will be made on Clincard, a reloadable debit card issued to the participant.

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