Study Protocol Effects of Exercise on Blood Flow Regulation in Adults with Down Syndrome (NCT04854122, approved 03/02/2022)

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Aims

To investigate the chronic response to exercise by conducting an exercise intervention in individuals with Down syndrome, to determine to what extent the improvements in work capacity will be explained by changes in central or peripheral blood flow regulation.

Aim 1: Determine if exercise training improves peripheral or central regulation of blood flow in individuals with Down syndrome compared to individuals with Down syndrome in a control group and to individuals without DS.
Aim 2: Determine if exercise training improves the cardiovascular autonomic profile in individuals with Down syndrome compared to individuals with Down syndrome in a control group and to individuals without DS.
Aim 3: Determine if exercise training improves gait and balance in individuals with Down syndrome compared to individuals with Down syndrome in a control group and to individuals without DS.

Lay language summary

Work capacity is an important predictor of declining health or physical function, and of mortality, and is commonly measured as peak oxygen consumption. Peak oxygen consumption is very low in individuals with Down syndrome, the most prevalent genetic cause of intellectual disability. Previous research suggests individuals with Down syndrome may experience a double disadvantage when they are exercising: they may not be able to increase cardiac output sufficiently and they may not be able to allocate adequate blood flow to the working muscles. The aim of this research proposal is therefore to investigate the responses in central and peripheral blood flow regulation and cardiac autonomic function to exercise training in individuals with DS. Additionally we investigate the effects of exercise on gait, balance, level of support and quality of life in individuals with DS.

Protocol and procedures

Design

This intervention study will be set up as a randomized controlled trial comparing the effects of a 12-weeks exercise program to 12-weeks of non-exercise usual care in participants with Down syndrome and to a control group of individuals without Down syndrome.

Description of study procedures

Familiarization sessions.

We involve parents/care givers in order to provide a supportive environment for participants and to enhance the parent/caregiver understanding of the research. Prior to study initiation, photographs and video clips of the laboratory and study equipment will be provided to help the participants become comfortable with the laboratory environment and equipment, and the informed consent information is sent to the participants and their parents/care-givers for their review. Familiarization of the data collection procedures for the participants with Down syndrome will be divided in two parts: they will practice and become accustomed with the treadmill and the equipment for the graded maximal exercise

test (first visit), and they will practice and become accustomed with the procedures for the hand grip exercise protocol and the LBNP (second visit).

Description data collection visits.

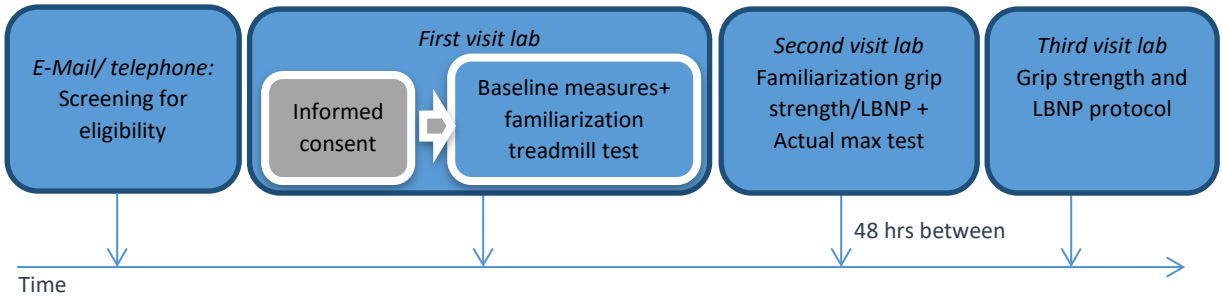
After discussing the study and potential questions either over the phone or in person, participants (and/or parents/caregivers for the group with Down syndrome) will complete a health history questionnaire and physical activity questionnaire to confirm eligibility. The Health History Questionnaire includes all the component of the new ACSM guidelines for the Preparticipation Health Screening for Exercise (Riebe et al. 2014, MSSE). Eligible participants with Down syndrome and their parent/care-giver will then provide written informed consent, or give verbal assent (for individuals with Down syndrome), and the eligible control participants will provide informed consent themselves. Participants are randomized directly after we have received their informed consent.

The first visit

For women of childbearing age, the first visit will include a urine pregnancy test using a test stick. Females will be studied during the first 3-5 days of menses or during the placebo phase if taking oral contraceptives, in order to control for hormonal variation. For participants with Down syndrome, the first visit continues with baseline measures (see below) and familiarization with the graded maximal exercise test protocol. If necessary, additional familiarization sessions will be scheduled for people with Down syndrome.

The second visit

We perform the graded maximal exercise test, the gait and balance test protocol, and familiarize the participant with the procedures for the third visit: the hand grip exercise protocol and the LBNP. The third visit we assess the peripheral blood flow during the hand grip exercise protocol without and with the LBNP (randomized order) (see figure below). At the end of the third visit, we will randomize the participants with Down syndrome to the exercise or the usual-care group. The participants randomized to the usual-care group will have the option to participate in the exercise program after completing their measurements at the end of the 12 weeks of usual care. For control subjects without Down syndrome, the first and second visit are combined. Their second visit is the same as the third visit for individuals with Down syndrome, however, they will not continue with any intervention, as their participation only includes baseline measurements.



Intervention. For the individuals with Down syndrome who are randomized to the exercise intervention: the exercise intervention will last 12 weeks and will consist of a supervised combined aerobic and resistance training program with a frequency of 3 days/week, and use a program comparable to Mendonca et al.[1, 2]. The program we will be using is specifically developed for individuals with Down syndrome based on the Mann Method PT Principles. The MMPT Principles focus on a progressive program of therapeutic activity (cardiovascular activity), therapeutic exercise (foundational strengthening and hip strengthening activities), and neuromuscular rehabilitation (visual/vestibular and balance activities) to address the unique needs of individuals with Down syndrome. This program has been developed and successfully implemented in Down syndrome activity centers across the country. The trainer will discuss with the participant what days/times work best for them. The exercise sessions will be offered as a remote exercise program, which means that all sessions will take place online through a HIPAA compliant Zoom platform. The trainer is part of the research team and will be a certified PT or a Personal Trainer certification with at least a bachelor degree in Exercise Science of Kinesiology. In the exercise sessions, the participant will work on their strength, balance and aerobic endurance. Each session consists of 10 min of strength exercises (Foundational Exercise), 10 min of Hip Strengthening, 10 min of Vestibular and Balance Exercise, and 20 min of Aerobic Exercise, and starts with a warming up and ends with stretching/cooling down. Each new exercise will be introduced in easy steps and practiced until the participant is comfortable executing it. After a 5 min. warm-up, the participant will perform 20 min of aerobic exercise at a heart rate of 65% of your maximum heart rate, which increases to 65-85% during the later weeks of the program. The resistance exercise part will include all major muscle groups, both upper and lower body, using multi- and single-joint exercises. Balance training will include exercises such as single leg stance and compliant surfaces with eyes open and closed, tandem stance with eyes open and closed, dual task walking, walking with change in speed, head turns, stepping over obstacles, external perturbations with eyes opened and closed, medicine ball toss. The caregiver does not need to be present during the exercise sessions.
The participants randomized to the control condition will continue with their usual daily routines for 12 weeks and then follow the same post-intervention data collection protocol as the intervention group, which is identical to the baseline collection. Participants in the control condition will be offered the opportunity to participate in the online exercise program after the post-intervention data collection, but this will fall outside the scope of this study.

Measurements

Work capacity, as measured by peak oxygen uptake with a graded exercise test on a treadmill, is the primary outcome variable. For the participants with Down syndrome, all measurements will be carried out pre- and post training under the same conditions and at the same time of day, for the control group without Down syndrome all measurements will be carried out as baseline measurements only.

Visit 1 for all participants

Baseline measures

Participants will be tested in a postprandial state (>3 h) on 2 separate days and will refrain from exercise 24 h before each test day. Participants will be also asked to refrain from drinking or eating caffeine and drinking alcohol on testing days. Participants who do not follow the requirements on study days will be

asked to come back another day. During the first visit, the participants will complete questionnaires about risk of falling (Short Falls Efficacy Scale-1), perform a cognitive function test on a laptop specifically designed for individuals with Down syndrome evaluating the following domains: Multitasking, Episodic memory, Executive function and Processing speed (Cambridge Neuropsychological Test Automated Battery (CANTAB)). After that, height, weight and circumferences will be measured. Body composition will be determined with a DEXA scan, to determine forearm composition and mass. Vascular health (arterial stiffness and central blood pressure) will be measured with the Complior Analyse device. Two-dimensional echocardiography (Hitachi Arietta 70 system, Tokyo, Japan) will be used to measure aortic root diameter, cardiac output, and stroke volume and end systolic volume at rest. In addition, clinical autonomic function tests (heart rate variability at rest and recovery after exercise, deep breathing, Valsalva maneuver, isometric exercise, active and passive orthostasis) will be evaluated during the first study visit. At the end of the first visit, all participants will be provided with an Actigraph and instructions to wear this device for 7 days.

Visit 2 for individuals with Down syndrome (added to visit 1 for individuals without Down syndrome)

Graded exercise test treadmill

Procedures

The participants will be familiarized with the graded maximal test protocol on the treadmill. The graded maximal exercise test begins with a submaximal horizontal walk on a motorized treadmill at a individualized comfortable speed, using a validated and reliable protocol for this population [3, 4]. Grade will be increased 2.5% every 2 min until a 12.5% grade will be reached. From this point, grade will be held constant whereas speed will be increased by 0.5 mph every minute until exhaustion. This may involve running at the later stages. The exercise test will be terminated when the participant can no longer keep up with the treadmill speed or shows signs of volitional fatigue. Heart rate, blood pressure and oxygen uptake (VO2) will be monitored continuously throughout the exercise and recovery [5]. The highest VO2 will be recorded as VO2peak if the respiratory exchange ratio is over 1.0 [6].

Measurements

Cardiac output will be measured using ultrasonography, by measuring the aortic diameter at the level of the valve form the peristernal long axis. Ascending aortic blood flow will be measured using continuous Doppler echocardiography using a pedoff probe in the suprasternal notch as we have previously described [7].

Gait and balance test protocol

This is a 30-min protocol evaluating different aspects of gait and balance with valid and reliable, easy, participant-friendly, quick and functional tests. For the Foot Posture Index the participant stands and the test instructor evaluates different aspects. This will be evaluated with and without the supportive footwear. The Timed Up and Go test is a timed test where the participant stands up from a chair, walk 10ft, turn around, walk back and sit down again, as fast as possible. For the Stair Climb test the participant is timed when climbing up a flight of 9 stairs. After that the participant performs a Functional Reach Test in the Virtubalance system, which electronically records how far the participant can reach

forward. After that the participant will be timed walking over a 30ft distance at a comfortable pace to determine Gait Speed (average of 3 attempts), and then will be walking that distance at his/her fastest walking pace, to determine Fast Walking Speed (average of 3 attempts). The participants will be executing these walking bouts on the GaitMat to evaluate spatial and temporal gait parameters and they will be recorded with 2D camera to determine lower extremity kinematics (knee and hip flexion, knee valgus/varus). To conclude, the participant will perform several standing balance tasks (Bertec Balance Measures) on a force platform. We will use a harness for safety, and the participant will be asked to lean in different directions following a target on a screen (evaluating the limits of stability), to maintain position while the platform moves in different directions (evaluating motor control) and to stand still for 3x 20 seconds with eyes open and 3x 20 seconds with eyes closed (evaluation sensory organization).

Visit 3 for individuals with Down syndrome (visit 2 for individuals without Down syndrome)

Hand grip exercise protocol

Procedures

Participants will be placed in a supine position with his/her lower body in the LBNP chamber, sealed at the waist. They will be instrumented with a 3 lead ECG to measure heart rate continuously and beat-to-beat blood pressure will be measured using finger plethysmography (Finapres NOVA, TNO Biomedical Instrumentation, The Netherlands).

Resting measurements will be conducted following a 10 min supine rest period. After the resting measurements, the participants are asked to squeeze the hand grip dynamometer with maximal force. This is repeated three times in total, with 1 minute of recovery in between. The highest is used as the participant's maximal voluntary contraction (MVC). Then the different parts of the hand grip exercise protocols start, with randomization of the order of Part 2B and 2C.

Part 2A: Diameters, blood flow and vascular conductance in the brachial artery will be measured in response to a short, single contraction of hand grip exercise with a hand grip dynamometer of 1 sec, at 15% and 30% of the participant's MVC. The peak blood flow within the first 4-6 cardiac cycles following the contraction will be used to determine the immediate contraction-induced vasodilation. These measures will be performed in triplicate, with 1 minutes of rest in between.

Part 2B: The vasodilatory response to dynamic hand grip exercise (2 sec rest, 1 sec squeeze for 4 minutes) at 15% and 30% of the participant's MVC (with 5 min of rest in between the different intensities) will be investigated. The change in diameters and blood flow of the brachial artery will be measured in the exercising arm with Doppler ultrasound in the last 30 sec of each condition.

Part 2C: The ability to vasodilate will be further challenged by adding a mild lower body negative pressure (LBNP) of -20 mmHg and performing the same dynamic hand grip exercise protocol and measurements as described in Part 2B. All measurements will be repeated during the last 30 seconds of the LBNP application. LBNP will be stopped if systolic blood pressure drops below 80 mmHg or if participants exhibit symptoms of pre-syncope. However, this protocol has been well tolerated in our pilot studies for participants both with and without Down syndrome.

Measurements hand grip exercise protocol

Forearm blood flow and vascular conductance will be measured in both the exercising and non-exercising arm using high definition ultrasound (Arietta 70, Aloka-Hitachi). The brachial artery will be imaged in dual mode allowing for simultaneous determination of artery diameter (B-mode) and flow velocity (Doppler mode). Blood flow will be determined from the following formula: Forearm Blood flow = (Mean blood velocity) x (Brachial Cross Sectional Area) x (60) and expressed as ml/min. Forearm vascular conductance will be determined by dividing forearm blood flow by mean arterial pressure. Forearm blood flow and vascular conductance will be normalized to forearm lean mass to account for differences in lean mass between individuals.

Muscle oxygenation and capillary blood flow will be measured with near-infrared spectography (NIRS), which is a simple, noninvasive method for measuring the presence of oxygen in muscle [8]. It can monitor changes in muscle oxygenation and blood flow during submaximal and maximal exercise. During exercise, the extent to which skeletal muscles deoxygenate varies according to the type of muscle, type of exercise and blood flow response[9]. Pulmonary oxygen consumption will be measured using an open-circuit breath-by-breath system (BIOPAC). Capillary blood flow can then be estimated by rearranging the Fick equation to solve for bloodflow (Q): Qcapillary = VO2muscle/ (arterio – venous O2difference), using the pulmonary oxygen uptake (VO2) and deoxyhaemoglobin (HHb) derived from NIRS to represent VO2muscle and (a −v)O2, respectively[10, 11].

Venous blood samples will be obtained from the participants with and without Down syndrome before and at the end of 5 min LBNP without hand grip exercise. A trained and licensed phlebotomist will obtain a total of 70 mL (60 mL before LBNP (approximately 4.5 tablespoons), 10 mL at the end of 5 min LBNP (approximately 0.75 tablespoons) blood sample via sterile venipuncture from the antecubital vein. The plasma will be analyzed for catecholamine concentrations (epinephrine and norepinephrine) with enzyme-linked immuno sorbent assays (ELISA kit). Additionally, the baseline samples pre- and post will be analyzed for C-reactive protein, TNF-alpha and interleukin-6 for inflammatory; insulin, glucose, leptin, adiponectin and lipid profile; and thyroid stimulating hormone as a marker for thyroid function. Based on previous research in Down syndrome on antioxidants, we will include superoxide dismutase-1 (SOD-1), catalase (CAT) and glutathione peroxidase-1 (GPX-1) [12, 13]. The generation of reactive oxygen species is increased in DS because of the mitochondrial dysfunction [14], but as the reactive oxygen species react too quickly to measure, the resulting oxidative products are used as proxy measures. We therefore include lipid peroxidation (measured by malondialhyde (MDA)), myeloperoxide (MPO, catalyzes the conversion of hydrogen peroxide to other reactive oxygen species), and oxidized low density lipoprotein (OxLDL). These oxidative products are all measurable in plasma or serum, and we will follow the specified ELISA procedures where applicable. We will also include a large-scale gene expression analyses for mitochondrial dysfunction and oxidative stress, which are located on chromosome 21, the chromosome affected by the trisomy 21 characteristic for individuals with DS. To this end, NextGen mRNA seq will be used to analyze the expression of SOD-1 and APP and a broad spectrum of expression pathways that are altered in the DS state. As an option, subjects may provide consent to bank any leftover blood samples upon study completion for use in future research about cardiovascular disease. The samples will be stored de-identified in the UNLV Translational Biomarker Unit.

Statistical Analysis

Descriptive statistics will be used to present baseline characteristics. A independent t-test will be used to test for differences in the change from pre to post between the exercise group and the control group. Additionally, a two-way mixed ANOVA will be used to evaluate condition (exercise and control) by time (pre-post) effects of the exercise program on the outcome measures. The a priori alpha level is set at 0.05.