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Effects of exercise intensity and nutrition advice on myocardial function in obese children and adolescents - a multi-centre randomised controlled trial study protocol

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We reported this protocol in accordance with the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) 2013 statement.

Keywords: Paediatric obesity, myocardial function, cardio-metabolic health, high intensity interval training, nutrition advice

Word count: 5,739

ABSTRACT

Introduction: The prevalence of paediatric obesity is increasing, and with it lifestyle-related diseases in children and adolescents. High intensity interval training (HIIT) has recently been explored as an alternate to traditional moderate intensity continuous training (MICT) in adults with chronic disease and has been shown to induce a rapid reversal of subclinical disease markers in obese children and adolescents. The primary aim of this study is to compare the effects of HIIT with MICT on myocardial function in obese children and adolescents.

Methods and analysis: Multi-centre randomised controlled trial of 100 obese children and adolescents in the cities of Trondheim (Norway) and Brisbane (Australia). The trial will examine the efficacy of HIIT to improve cardiometabolic outcomes in obese children and adolescents. Participants will be randomised to (1) HIIT and nutrition advice, (2) MICT and nutrition advice or (3) nutrition advice. Participants will partake in supervised exercise training and/or nutrition sessions for 3 months. Measurements for study endpoints will occur at baseline, 3 months (post-intervention) and 12 months (follow up). The primary endpoint is myocardial function (peak systolic tissue velocity). Secondary endpoints include vascular function (flow-mediated dilation assessment), quantity of visceral and subcutaneous adipose tissue, myocardial structure and function, body composition, cardiorespiratory fitness, autonomic function, blood biochemistry, physical activity and nutrition. Lean, healthy children and adolescents will complete measurements for all study endpoints at one time point for comparative cross-sectional analyses.

Ethics and dissemination: This randomised controlled trial will generate substantial information regarding the effects of exercise intensity on paediatric obesity, specifically the cardiometabolic health of this at-risk population. It is expected that communication of results will allow for the development of more effective evidence-based exercise prescription guidelines in this population while investigating the benefits of HIIT on subclinical markers of disease.

Trial Registration: NCT01991106

Strengths and limitations of this study

- To our knowledge, this multi-centre trial is the first to use a combined exercise and nutrition programme to examine the effect on myocardial function in obese children and adolescents. It also one of few trials to explore the efficacy and feasibility of high intensity interval training in this population.
- Strengths of this multi-centre trial lie in the rigor of the twelve-week exercise and nutrition intervention. The majority of exercise sessions will be supervised to ensure that the correct exercise intensity is achieved at all times.
- Extensive resting and exercise outcome measures will be performed at both trial centres. Several measures are sensitive to small but important longitudinal changes and are novel in paediatric obesity.
- Like any paediatric longitudinal trial, challenges remain around growth and maturation over the trial period, which have the potential to confound study endpoints. While the primary study endpoint is related to cardiac growth, any changes in cardiac size over the one-year program will be accounted for using normalisation methods.
- Maturational changes will also be accounted for through Tanner Stages of puberty however these may be subject to self-report error.

INTRODUCTION

Paediatric obesity rates have increased over the last two decades, and is now prevalent in 3-15% of the paediatric population according to the International Obesity Task Force definition of obesity [1]. More than 60% of obese children will become obese adults with a significantly increased risk of developing non-communicable diseases including cardiovascular diseases, cancer and type 2 diabetes mellitus (T2DM). In 2012, these diseases accounted for 63% of deaths worldwide [2] and 77% of disease burden in Europe [3]. Indeed, a childhood and adolescent body mass index (BMI) above the 95th percentile is a strong predictor of adult mortality rates [4]. Furthermore, children and adolescents who had a baseline BMI between the 85th and 95th percentiles, defined as overweight, had a 30% increase in all cause mortality. This increased risk of death was independent of their adult BMI [4].

Obese children and adolescents may show abnormal myocardial function when assessed through resting and stress echocardiography [5-9]. Echocardiographic techniques such as tissue Doppler imaging are able to detect subclinical heart disease [10]. Previous investigations have shown significantly reduced Doppler tissue velocities in obese youth compared to lean, healthy age-matched control participants [11]. In particular, peak systolic tissue velocity (s') closely reflects left ventricular contractility [12], which can be improved with short-term exercise training [13].

Increased BMI or overweight in early life (1-9 years) is associated with coronary artery disease [14] and it is acknowledged that atherosclerotic processes begin in childhood [15]. Impaired vascular function determined by flow mediated dilation (FMD) of the brachial artery has been observed in a number of obese paediatric studies. Visceral adipose tissue is increased in obesity which results in a greater release of bioactive mediators [16]. These influence the function of adipose

tissue and contribute to chronic disease, having a substantial impact on insulin sensitivity, inflammation and subsequent risk for dyslipidemia, T2DM and atherosclerosis [17].

Current pediatric guidelines for treating paediatric obesity recommend lifestyle modification to encourage family based behavior change leading to a reduction in energy intake and increase in physical activity [18,19]. The current treatment of pediatric obesity appears to have a low success rate, most likely due to the heterogeneous causes of obesity. Several recent meta-analyses and reviews have demonstrated that the effectiveness of pediatric obesity treatment is limited [20-22]. The response to treatment differs substantially between clinical centers and treatment success appears to be age-related. A large European registry study showed significant treatment effects following a variety of lifestyle interventions including exercise programs, nutrition education, psychological intervention and parental education, in less than 10% of participants over two years of follow-up [23]. Furthermore, the treatment was least effective in participants older than 12 years old. However, the low success rate was in part accounted for by a high dropout rate. Two recent reviews suggest that lifestyle and exercise interventions in obese children and adolescents can lead to improvements in anthropometric and cardiometabolic outcomes. These reviews are not inclusive of several important outcomes such as myocardial and vascular function, visceral adipose tissue or cardiorespiratory fitness [20,24].

High intensity interval training (HIIT) has recently been explored as an alternate exercise to moderate continuous intensity training (MICT) in healthy adults as well as those with chronic disease. HIIT involves a short bout of exercise at a high intensity, interspersed by recovery periods in preparation for the next bout. HIIT has resulted in improved health markers in adults with cardiometabolic disease [25] while

demonstrating time-efficiency [26]. Four studies to date have examined the physiological effects of HIIT, compared to MICT in obese children and adolescents [11,27-29]. Children and adolescents expressed increased enjoyment during high intensity interval training [26] and the stop-start nature of HIIT may reflect play-based activities traditionally observed during childhood. We have previously shown that HIIT in obese adolescents can almost normalise cardiac function to that observed in lean counterparts [11] however the pilot trial had a small sample and did not include comparative treatments. We therefore wish to examine the physiological efficacy of HIIT compared to MICT in a multi-centre randomised controlled trial, thereby improving the current literature and informing the treatment options for paediatric obesity.

OBJECTIVES

The primary aim of this randomised controlled trial is to compare the effectiveness of three interventions: HIIT and nutrition advice, MICT and nutrition advice, or nutrition advice alone on myocardial function in obese children and adolescents. It is hypothesised that HIIT will be superior in improving myocardial function compared to MICT. Secondary outcome measures will include vascular function, body composition (total, visceral and subcutaneous adipose tissue depots and lean muscle mass), cardiac structure, cardiorespiratory fitness, autonomic function, serum fasting lipids and insulin sensitivity, biomarkers of inflammation, satiety and oxidative stress markers, physical activity levels and nutrition. Assessments will occur at baseline, after 3 months of supervised training and at 12 months (after 9 months of home-based training). For the 9 months of home-based training, participants will be randomised to 1) monthly supervised exercise or 2) home-based exercise only. Therefore the 12-month assessment also aims to determine

the amount of supervision required to maintain training habits by comparing these two groups.

METHODS AND ANALYSIS

Study setting

This is a multi-centre randomised controlled intervention trial. The study centre is located at The Norwegian University of Science and Technology (NTNU), Trondheim, Norway. The other centre is at The University of Queensland (UQ), Brisbane, Australia. Testing and training will take place in the university research laboratories at these institutions and hospital outpatient settings (St. Olav's Hospital in Trondheim & The Wesley Hospital in Brisbane).

Participants and eligibility criteria

The study cohort will include 100 obese and 100 lean, healthy control children or adolescents aged between 7-16 years old. Obesity in this population will be defined as a BMI equal to or greater than the 95th percentile (age and sex specific) [30]. Study exclusion criteria include hypertension (defined as blood pressure above the 95th percentile for systolic or diastolic values), any history or evidence of heart disease and/or an abnormal resting or stress echocardiography which indicates it would be unsafe to participate, any chronic disease e.g. chronic asthma, kidney disease, diabetes, current smoking habits, or an orthopaedic/neurological disorder that may limit ability to exercise, diagnosed attention deficit hypersensitivity disorder and use of steroid medications. Conditions not specifically mentioned above may serve as criteria for exclusion at discretion of the clinical site. Furthermore, if medical conditions become apparent during testing or training in participants, medical advice will be sought and the intervention may be discontinued in the individual. Lean healthy control participants must have an age and sex specific BMI in the healthy

range (5^{th} – 85^{th} percentile) to be included [30]. Study exclusion criteria are identical in lean healthy control participants. The lean healthy control participants will not partake in an intervention but will be assessed for cross-sectional comparative analyses.

Interventions

A randomised block design will be used. Obese participants will be randomised to one of three groups and will be stratified according age and sex. Lean participants will be stratified to the same groups but no intervention will be administered (Figure 1).

Exercise Protocol

The exercise intervention involves a combination of supervised and unsupervised exercise training sessions. Participants will attend at least two, up to three supervised training sessions each week for twelve weeks. If participants choose to attend two supervised sessions, they will be required to complete a third unsupervised exercise session at home. Following the three-month supervised period, participants in each of the two exercise groups will be randomised to 'monthly supervised exercise' or 'home-based exercise only' from 3 to 12 months. During this time, participants in the HIIT and MICT groups will be asked to complete three unsupervised training sessions each week for nine months and the 'monthly supervised exercise' group will be asked to attend once-monthly supervised training sessions at the clinical site.

Supervised exercise training (HIIT and MICT) will consist of walking or running on a treadmill, or cycling on a stationary bike based on participant preference. During the unsupervised exercise session the mode can vary. Heart rate, rating of perceived exertion (Pictorial Children's Effort Rating Table - PCERT), and exercise

mode will be recorded in a training booklet. Participants who wish to complete an unsupervised session each week will be provided with a separate booklet, which will be kept alongside the clinic version. Each participant will also receive a training booklet for the follow up period allowing them to record details of each session completed.

High intensity interval training

Participants randomised to HIIT will perform a 10-minute warm up at 60-70% of maximal heart rate (HRmax). Following this, they will walk, run or cycle at 85-95% of their maximal heart rate for four, 4-minute intervals, with 3 minutes of active recovery (50-70% of HRmax) between the intervals. Participants will perform a 5-minute cool down period at the end amounting to a total exercise time of 40 minutes.

Moderate intensity continuous training

Participants randomised to the MICT group will walk, run or cycle continuously at 60-70% HRmax for 44 minutes to approximate the average energy expended by the HIIT group as previously calculated by our research group [31].

Nutrition advice

HIIT, MICT and nutrition groups will receive eight to ten, 20-minute individual nutrition sessions with a dietitian over the twelve-month period. Content of the sessions will include healthy food choices, portion sizes and regular meal times. The nutritional advice given will reflect current Norwegian and Australian eating guidelines and will be location specific [32,33]. The nutrition group will not be provided with any prescribed supervised exercise.

Outcomes

Measurements will take place at baseline, 3 months (end of the supervised exercise intervention period) and at 12 months follow up.

Participant preparation guidelines

Participants will be informed of preparation requirements, which will be checked prior to the assessments.

Stress echocardiography & cardiorespiratory fitness testing

Assessment of myocardial function and structure, and maximal oxygen uptake will require participants to refrain from eating heavy meals up to two hours before the testing session and avoid any foods containing caffeine for this time period.

Vascular function, body composition and blood collection

Assessment of vascular function, body composition (BodPod) and blood collection will be completed in a fasted state (8-12 hours) and participants will be instructed to avoid caffeine, vitamin C, alcohol, drugs, stimulants and medications for this time period. Additionally, participants must refrain from intense exercise for 48 hours prior to testing. To avoid dehydration, participants will be instructed to drink at least 0.5L of water before attending the examination.

Myocardial function (rest and stress echocardiography)

Primary Outcome Measure: Peak systolic tissue velocity (S') at rest

Secondary Outcome Measures: S' (during exercise, both ventricles), S' (rest, right ventricle), peak diastolic tissue velocities (both ventricles), tricuspid annular plane systolic excursion (TAPSE), global strain and strain rate, aortic flow, cardiac dimensions.

A full resting echocardiogram will be performed with a Vivid 7/E9 ultrasound machine (GE Vingmed Ultrasound, Horten, Norway) using a phased-array transducer

(GE M3S). Three cine loops from the three standard apical planes (four-chamber, two-chamber and long-axis) and the right ventricle will be recorded in grey scale harmonic mode and tissue Doppler mode simultaneously. LV standard Doppler echocardiographic indices will be measured and body surface area (m^2) will be used to normalize cardiac dimensions for differences in body size. Mitral annulus excursion, pulsed wave tissue Doppler velocities; peak systolic (S'), peak early diastolic (e') and peak late diastolic (a') will be measured at the AV-plane level in 4-chamber and 2-chamber view and a mean of the 4 points will be used. Right ventricle function standard Doppler echocardiographic indices will be measured and TAPSE, S', e', a'. Deformation (strain) and deformation rate (strain rate) will also be analysed by speckle tracking (2D strain) and tissue Doppler imaging.

Following the resting echocardiogram, individuals will exercise on a stationary cycle ergometer in an upright position. The exercise protocol will start at an intensity of 25W with 25W increments every three minutes until participants have attained their maximum heart rate or are no longer able to maintain a constant cadence. Recordings will be made at baseline and peak assessing apical 4-chamber and 2-chamber in B-mode and tissue Doppler as well as mitral and aortic flow. A three lead electrocardiogram (ECG), blood pressure and ratings of perceived exertion (PCERT) will be monitored and recorded at the end of each stage.

EchoPAC™(GE Vingmed Ultrasound AS, Horten, Norway) will be used for all echocardiographic analysis by an investigator blinded to the group allocation of the subjects.

Visceral, subcutaneous and total abdominal adipose tissue (MRI)

At the Brisbane site, visceral, subcutaneous and total abdominal adipose tissue adipose tissue will be measured using a 1.5 Tesla magnetic resonance imaging (MRI)

system (Siemens Symphony Sonata, Siemens, Erlangen, Germany) equipped with a 6 channel body matrix coil and a 6 channel spine coil. Subjects will be positioned supine inside the magnet and transversal images will be acquired using TRUFISP (true fast imaging steady state precision) technique with breath-hold (repetition time = 3.76 ms; echo time = 1.88 ms; flip angle 75deg; matrix = 220 x 256; rectangular field of view (FOV) = 400 mm x 400 mm; slice thickness = 8mm; 14 slices; acquisition time = 12 sec). We will acquire 14 axial slices, 8mm thick centred over the umbilicus during breath-hold using a Dixon technique.

At the Trondheim site, visceral, subcutaneous and total abdominal adipose tissue will be measured using a 3 Tesla MRI system (Siemens Skyra; Siemens, Munich, Germany) equipped with an 18 channel body coil and a 32 channel spine coil. Subjects will be positioned supine inside the magnet and transversal images will be acquired using T1-weighted Dixon vibe sequences with breath-hold (repetition time=4,04ms; echo time=1.3ms and 2.50ms; flip angle=9deg; matrix=320 x 256; rectangular field of view (FOV)=380mm x 309; Slice thickness=3mm; 52 slices; Number of averages=1; Band width=1120Hz/pixel; GRAPPA parallel imaging acceleration factor 2, acquisition time=17 sec). If the given FOV is too small to cover the whole subject, the FOV is increased sufficiently before scanning. The Dixon sequences will be acquired twice to assure a set of successful images.

The MRI scans will be exported and anonymised. Images will be converted to NIfTI format and analyzed using in-house software developed in MATLAB (TheMathWorks, Inc, Natick, MA). Five consecutive slices at and above the L4/L5 vertebral disc, with localizing images used to confirm the level, will be selected for quantification of visceral and subcutaneous fat in each participant. Two regions of interest (ROIs) will be manually drawn on the images by a trained radiologist. One

ROI will delineate the subcutaneous compartment and the second ROI will delineate the intra-abdominal and retroperitoneal areas together to quantify visceral adipose tissue. Visceral and subcutaneous adipose tissue will be summed to quantify total adipose tissue. The area of the fat inside each ROI will be calculated by counting the number of pixels with intensities above a selected threshold and multiplied by the pixel area. Slices will be manually adjusted for threshold intensity in order to compensate for lack of uniformity between slices. Values calculated from the five slices will be averaged to provide a mean area (cm^2).

Vascular function (flow mediated dilation)

Endothelial function of the brachial artery will be measured via flow mediated dilation using high-resolution vascular ultrasound (12-14 MHz ultrasound- Doppler probe, Vivid 7 system/Vivid I system; GE Vingmed Ultrasound AS, Horten, Norway) according to current guidelines [34]. Participants will lie supine for ten minutes in a quiet, dark environment prior to commencement of the procedure. A transducer will be placed against the brachial artery and following image optimisation, baseline images will be recorded for at least 30 seconds in live duplex mode (simultaneous B-mode diameter and pulsed-wave Doppler velocity signals). A blood pressure cuff will then be placed distal to the region of interest and inflated for five minutes at 200mmHg. This will reduce blood flow to the hand. Upon cuff release, 10 beat cine-loops will be recorded in duplex mode (B-mode diameter and pulsed-wave Doppler velocity signals) at 10s, 30s, 60s, 90s, 120s, 150s and 180s (UQ), or continuously for 3 minutes (NTNU) to measure the change in the diameter of the artery following increased blood flow and shear stress to the vascular wall. Data will be assessed by custom-made automated edge-detection software, which is independent of

investigator bias. Brachial artery diameter and shear rate will be quantified from the ultrasound studies to assess flow-mediated dilation.

Cardiorespiratory fitness

Maximal oxygen uptake (VO_2max) will be measured during uphill treadmill walking or running using expired air gas analysis (Metamax 3B, Cortex Biophysik, GmbH, Leipzig, Germany or Jaeger Oxycon Pro, CareFusion, Hoechberg, Germany). An incremental, ramp protocol will be used for all participants. A four minute warm up at 4km/h and 0% grade will precede the test however the speed can be modified to match the preferred walking speed of the participant. A walking or running protocol is available depending on participant preference and fitness.

The walking protocol consists of one minute stages where speed is set at preferred walking speed and gradient is increased by 2% each minute. If the participant reaches a steep gradient (this will be adjusted by researchers depending on age and height of participant: <12yrs ~12%, ≥12 yrs ~16%), speed will be increased by 2km/h each minute thereafter.

The running protocol consists of one-minute stages where gradient is set at approximately 10% (this will be adjusted by investigators depending on age and height of participant) and speed is increased by 1km/h each minute. If the participant reaches a speed that can no longer be safely increased, gradient will be increased by 1% each minute thereafter.

A levelling off of oxygen uptake (VO_2) despite increased workload and respiratory exchange ratio (RER) ≥1.05 will be used as criteria for VO_2max.
A levelling off (plateau) in VO_2 will be defined using 30-second epochs. If the VO_2 increase is $\frac{<150 ml O_2/min}{Body\ mass}$ with an increase in workload, then a plateau is assumed. If the participant has reached a plateau and the RER criterion has been satisfied then the

two highest consecutive 30-second values will be averaged to obtain the VO_2max value. If the participant has not reached a plateau, VO_2peak is determined by using the average of the two highest values attained. It is highly likely that most participants will not reach a VO_2max and therefore group values will be reported as VO_2peak.

Heart rate will be measured continuously during the test (Polar, Polar Electro, Kempele, Finland), to define HRmax.

Heart rate variability

Participants will lie supine for ten minutes in a quiet, dark environment prior to commencement of the procedure. Participants will be asked to lie as still as possible for five minutes while an ECG trace is monitored and recorded for calculation of heart rate variability. RR intervals obtained from the ECG will be processed using Kubios HRV (University of Eastern Finland, Finland).

Body composition

At the Brisbane site, dual energy x-ray absorptiometry (DXA) will be used to determine body composition (adipose tissue and lean muscle mass). This will require the participant to lie motionless while an x-ray of their entire body is taken using the DXA scanner (Hologic, QDR Series, MA, USA). The duration of a whole body scan is 7 minutes.

At the Trondheim site, a BodPod (COSMED, Rome, Italy) will be used to determine body composition (adipose tissue and lean muscle mass). Participants are required to be fasted (8 hours) and will be tested in minimal clothing (underwear only). The procedure takes 15 minutes in total with 5 minutes spent in the BodPod.

Blood biochemistry

Venous blood samples will be collected from a superficial antecubital vein according to standard phlebotomy procedures. Samples will be collected into three

vacutainers containing EDTA, fluoride oxalate and clot activators. Vacutainers will be stored on ice, or left to clot at room temperature for at least 30 minutes (serum samples). Two aliquots of whole blood will be pipetted out and following this, samples will be centrifuged at 2500 rpm, 4 degrees Celsius for 10 minutes. Plasma/serum will be aliquotted and stored at -80 degrees Celsius for later analysis. Samples will be analysed for lipids (total cholesterol, LDL, HDL, triglycerides), glucose and insulin (to establish insulin resistance and beta cell function using HOMA-IR) and CRP using spectrophotometry (Cobas Mira, Roche Diagnostics, Australia). Insulin resistance and beta cell function will be calculated using the HOMA model (based on fasting glucose and insulin concentrations) where $HOMA-IR = \frac{Glucose\ x\ Insulin}{22.5}$ and $HOMA-\beta = \frac{20\ x\ Insulin}{Glucose-3.5}$ %. Satiety hormones (ghrelin, leptin, peptide YY, obestatin), inflammatory markers (TNFα, IL-6, IL-10, PAI-1), adiponectin and si-CAM-1 will be measured using specific ELISA kits (R&D systems, Inc., Minneapolis, MN, USA). Total nitrite concentration will be quantified using a commercially available assay for nitric oxide (NO_2-) detection (R&D systems, Inc., Minneapolis, MN, USA). Oxidative stress and antioxidant status will be measured using the following methodologies. Total F-2 isoprostanes will be extracted and analysed using a method developed by the Brisbane laboratory [35]. The laboratory coefficient of variation for this assay is 4.5%. Protein carbonyls will be analysed using adapted methodology from Levine et al. (1990) [36]. The laboratory coefficient of variation for this assay is 11.9%. Plasma glutathione peroxidase (GPx) activity will be measured spectrophotometrically (Cobas Mira, Roche Diagnostics, Switzerland) via the oxidation of NADPH to NADP by modifying methods [37,38]. The laboratory coefficient of variation for this assay is 2.4%.

Physical activity and nutrition measurements

Accelerometry will be used to measure physical activity at baseline and at the three-month assessment. Participants will be asked to wear an accelerometer for seven days (Brisbane: ActiGraph, Florida, USA and Trondheim: SenseWear, BodyMedia, Inc., Pittsburgh, USA). To be included in the analysis, participants will be required to have a minimum of four valid days, one of which must be a weekend day. Participants in Brisbane will be asked to wear the ActiGraph monitor during waking hours, except for when sleeping or during water-based activities. Participants will also be asked to keep a brief log to record wake/sleep times and any time the monitor was removed for >10 minutes (e.g. sleep, shower etc.). The ActiGraph accelerometer will be initialised to sample at 30Hz and data will be aggregated in 15-second epochs. To be considered a valid day, there must be a minimum of 10 hours of wear time and non-wear time criteria will be 60 minutes or more of consecutive zeros. Accelerometer cut points previously validated in a paediatric population [39] will be used to determine average time per day spent in light physical activity, moderate physical activity and vigorous physical activity. ActiGraph data will be analysed using ActiLife software (version 6, Florida, USA). Participants in Trondheim will be asked to wear the SenseWear armband for 24 hours each day. The device will be removed during water-based activities. To be considered a valid day, at least 85% of a 24-hour day must be recorded and time spent in light physical activity (1.6-2.9 METS), moderate physical activity (3-5.9 METS) and vigorous physical activity (>6 METS) will be determined. SenseWear data will be analysed using BodyMedia (version 8.1, Pittsburgh, USA).

Participants will also complete a physical activity questionnaire designed by the Norwegian Directorate of Health with content focusing on total physical activity, physical activity at school and home, and weekday/weekend screen time [40].

Food record booklets will be given to participants and a three to four-day record will be requested (must include one weekend day). Food records will be analysed using either FoodWorks (Xyris Software, Australia) for the Australian cohort or using the food database KBS AE-07 and KBS software system (KBS, version 4.9, 2008, Department of Nutrition, University of Oslo, Norway) for participants in Norway.

Other measures

Height, weight, waist (WC) and hip circumferences (HC) and blood pressure will be measured using standard approaches [41].

Participant timeline

Figure 2 illustrates the schedule for measurement of outcomes and the intervention.

Sample size

The sample size was calculated for a one-way ANOVA analysis comparing the mean change in S' between the three groups from pre to post intervention. Although the data will be analysed using a linear mixed model (LMM), a simplified calculation of sample size for a one-way ANOVA provides a conservative estimate of the sample size required for the LMM. Simulations run by our research group support this assumption. For calculation of sample size, the clinically meaningful difference in means was set to 1cm/s, using the values 9.5, 10 and 10.5 cm/s for the nutrition, MICT and HIIT groups, respectively. The standard deviation, assumed to be equal for all groups, was set to SD=0.9 cm/s [11]. To obtain a power of 0.80 for the overall test of differences in means, using a significance level of 0.05, 17 individuals are required in each group. A further 40 individuals are required to account for the four stratification groups (Figure 1) included in all statistical analyses. To account for 15% dropout, a total of 105 individuals are required to enter the intervention. In order for

the lean control group to be closely matched in age, sex and sample size, 105 lean participants are required for assessment at a single time point.

Recruitment

A variety of recruitment strategies will be employed at each site to achieve adequate participant enrolment and reach target sample size. At the Trondheim site a regular advertisement will be placed in the local newspaper. In addition, advertisements will published on the university and hospital websites and flyers will be distributed at strategic locations. Every six months, local health nurses and medical centres will be informed about the study. A website will be set up with a linked preliminary screening tool and program contact form. A video blog about childhood obesity research will be used for media outlets and publicity through newspapers and television. Social media such as Facebook will also be used. Similar strategies will be used at the Brisbane site. An advertisement for the study will be placed in the university staff newsletter and flyers will be placed around the campus. Schools in a 30km radius will be emailed with a newsletter advertisement. Health & fitness centres and medical centres within a 15km radius will be asked to advertise the program on a noticeboard. A website will be set up with a linked preliminary screening tool and program contact form. Google AdWords will be employed as a continuous recruitment pathway strategy with approximately 10-15 clicks resulting in website views expected each day. Local dietitians and paediatricians will also be contacted for referrals into the program. Finally, media outlets will be contacted for newspaper and television coverage.

Assignment of interventions

Allocation sequence generation

Computer generated random numbers will be used for allocation sequence generation with stratification completed according to age and sex (see Figure 2.0). The four stratification groups will be:

1) Females 7-10 years
2) Males 7-12 years
3) Females 10-16 years
4) Males 12-16 years

Allocation concealment will be implemented using central randomisation through a web-based program that will generate the allocation sequence. A study investigator at each centre will enter the details of eligible obese participants and the web-based program will provide the group allocation for the intervention. The investigator will then inform the parent or guardian. Lean participants will also be registered and stratified using the web-based program.

Blinding

As this is an exercise intervention, trial participants cannot be blinded to group assignment. However, outcome assessors, and data analysts will be blinded to intervention assignment. Outcome assessors of the primary outcome are independent of the clinical centre and will not interact with participants outside of the assessments. Participants will be asked not to divulge their group allocation during testing visits. All data is stored using participant ID number only and data analysis that is subject to investigator bias will occur without knowledge of intervention assignment.

Data management and analysis

Data management

Double data entry will be administered at each site to ensure data quality. Data will be stored in a re-identifiable format (participant numbers only). A password-protected sheet will enable the participants' numbers be linked to names when required.

During and after the research project, data on paper will be kept in a locked filing cabinet. Electronic data will be stored on password-protected computers or external hard drives with access granted only to members of the research team. Tissue samples will be identified by participant number only and will be stored in a secure locked area.

Information collected will be disposed of ten years after study completion. Paper documents will be shredded and disposed of while electronic information will be erased.

Statistical methods

An intention to treat analysis will be used. A per protocol analysis will also be conducted where completion of 80% of the 3-month intervention is required. Data will be analysed with SPSS Statistics (IBM, NY, USA), Stata (TX, USA) and R (R Core Team, Vienna, Austria). Normality of data will be checked using one or more normality tests. Descriptive statistics will be computed for variables of interest and continuous data will be reported as means and standard deviations if data is normally distributed. Non-continuous and non-normally distributed data will be reported as frequencies, medians and interquartile ranges.

Statistical analysis comparing between group differences (3 groups) following the intervention will be conducted using a linear mixed model (LMM). This technique calculates between-group and within-in group differences (from baseline to post-intervention) within the same model. A linear mixed model is also able to adjust for stratification variables (age, sex) and for a site effect if present. In order to examine the effect of supervision during the nine-month follow up period (from three to 12 months), an LMM will be used again with supervision included as an additional explanatory variable in the model.

To compare between-group differences in binary categorical data following the 3-month intervention, a generalised linear mixed model (GLMM), which accounts for time correlations, will be used.

Monitoring

Harms

If a participant develops a medical or surgical illness during the study, the Data Monitoring Committee in cooperation with the participant's general practitioner will ascertain continuing or resuming participation in the intervention. In the event of a medical emergency occurring at the clinical sites, the study staff will undertake, under direction of the principal investigator or designated staff, all necessary supportive medical care.

All adverse events (AE) will be reported between the first trial-related study procedure and the last during study intervention. Medical events that occur between the signing of the Informed Consent Form and the final study-related procedure will be documented in the medical history.

Participants should voluntarily report any AE's or in response to general, non-directed questioning (e.g., "How has your health been since the last visit?"). For each AE volunteered by the participant, the investigator will obtain all the information required to complete the AE documentation. All AE's regardless of seriousness, severity or presumed relationship to the study will be recorded using medical terminology in the source document and on the case report form. Safety related events will be reported in a timely fashion as required by the Data Monitoring Committee and the Ethics Committees responsible for the study.

ETHICS AND DISSEMINATION

Research ethics approval

The investigation protocol and procedures have been approved at each clinical site. At NTNU, Norway, the Regional Committee for Medical and Health Research Ethics has approved this project (reference number 2009/1313-4). At UQ, Australia, The University of Queensland Human Research Ethics Committee (reference number 2013000539), The Mater Hospital Human Research Ethics Committee (reference number HREC/13/MHS/119/AM01) and Uniting Care Health Human Research Ethics Committee (reference number 1324 has approved this project.

Consent and assent

Participants and their parents will provide informed consent after the principal investigator has briefed them on the study and answered questions. Two separate informed consent sheets will be signed with content adjusted to suit a paediatric population (participant and parent/guardian signs one while the other is signed by the participant only).

Confidentiality

Information collected directly from participants will be in a re-identifiable form and any information collected for, used in, or generated by this project will not be used for any other purpose. The site principal investigator and associated research personnel such as the study dietitian will have access to information.

Declaration of interests

There are no competing interests for principal investigators of the overall trial and/or individual study sites.

Dissemination policy

Results of this clinical trial aim to be disseminated through peer-reviewed journal articles, conference abstracts and presentations, as well as media publications.

It is hoped that results of the clinical trial will inform future paediatric obesity management strategies.

DISCUSSION

Reduced physical activity and poor nutrition are the main causes of obesity in children and adolescents. A large proportion of the paediatric population does not meet exercise guidelines and energy intake in obese children is often larger than in healthy weight children [42,43]. Consequently, paediatric obesity has increased steadily in several countries over the last thirty years [44-46]. Current worldwide data suggest that only 5-50% of children and adolescents are meeting current exercise guidelines [47-53]. Furthermore, obese children are less physically active compared to healthy weight children [54,55] and spend more time in sedentary activities [56,57]. Low levels of leisure time physical activity are associated with an increased risk of cardiovascular and metabolic diseases [58]. Therefore, encouraging all children to increase their levels of physical activity and reduce their sitting time could help to avoid excess weight gain and associated health risks [59]. We therefore are striving to investigate a time efficient form of exercise, which could potentially induce physiological changes and reduce cardiovascular risk factors in obese children and adolescents. The importance of nutrition in this population stipulated that the exercise interventions were combined with nutrition advice, and compared to a nutrition advice only group as well.

This clinical trial involves the largest known cohort of obese children and adolescents in a HIIT intervention to date. Valuable information about the effects of exercise intensity on cardiac and vascular structure and function, body composition, cardiorespiratory fitness, biochemistry markers, physical activity, nutrition and quality of life in obese children and adolescents will be gathered. Importantly, this

trial will contribute to the currently small, but important, body of evidence exploring cardiac function during exercise. While resting function may remain normal in paediatric obesity, stress echocardiography may unmask subclinical cardiac disease [60] and lead to better patient management and outcomes.

Dissemination of the knowledge gained from this trial is expected to inform physical activity and exercise guidelines for this specific population in an attempt to dampen the impact that obesity has on physiological systems as well as reduce risk factors for future development of comorbidities such as T2DM and heart disease.

Contributorship statement: Each author: (1) made substantial contributions to conception and design; (2) assisted in drafting the article and provided important intellectual content and (3) gave final approval of the version to be published. Given the multi-disciplinary nature of this trial, specific contributions were made by each author. CI and AT conceptualised the trial and drafted the original study methods. KD, JC and CI refined study methods and will be the project managers of this multi-centre research trial. CI and PC expertise lie in assessment of myocardial function and structure while DJ and MSH specifically assisted with non-invasive methodologies for assessing vascular health. MH and EH designed the protocol for assessment of visceral and subcutaneous fat, while SK, MH and EH developed analysis methodologies for this outcome. PD and SH expertise lie in paediatric nutrition and therefore contributed to design of the nutrition intervention. GL has extensive experience in paediatric endocrinology, and paediatric obesity lifestyle interventions. GL assisted with general study design and identifying blood biochemistry variables of interest. SJ assisted with selecting an activity monitor device and with study design to collect and analyse physical activity levels. AT and KD designed the methods for the maximal oxygen consumption test. TR specifically contributed to biostatistics of the trial including sample size and power calculations, and statistical analysis methods.

Competing interests statement: Dr. Coombes reports grants from Coca Cola, personal fees from Tolmar Pharmaceuticals, personal fees from Novo Nordisk Pharmaceuticals, outside the submitted work. The remaining authors have read and understood BMJ policy on declaration of interests and declare that they have no competing interests.

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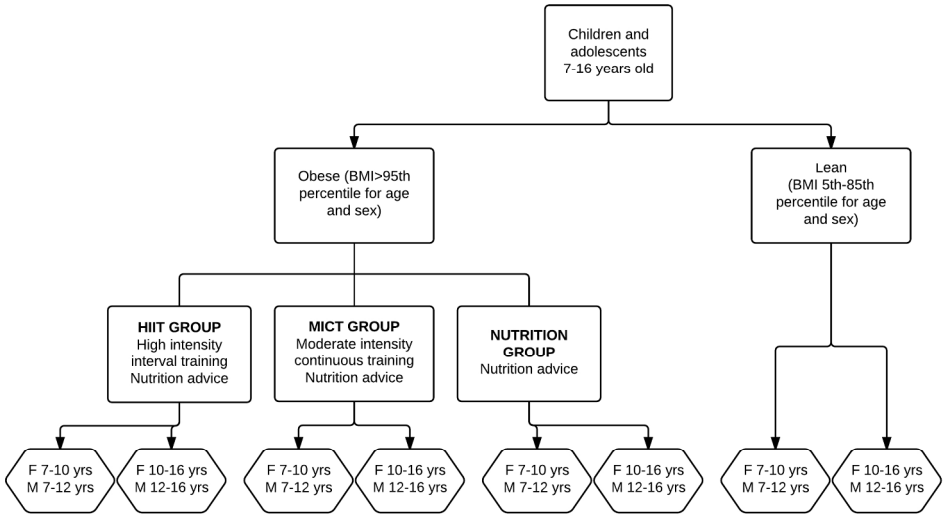

Figure 1: Intervention groups/ Stratification
200x114mm (300 x 300 DPI)

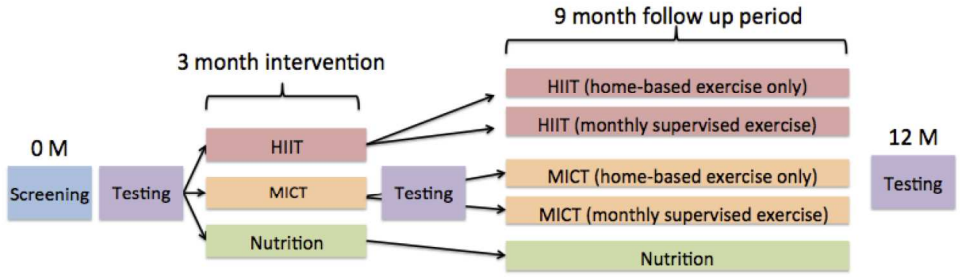

Figure 2. A schematic illustrating a time schedule for enrolment, intervention and assessment of obese participants.
297x209mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	26
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction

Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	____4____
	6b	Explanation for choice of comparators	____5_____
Objectives	7	Specific objectives or hypotheses	____6_____
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	____7_____

Methods: Participants, interventions, and outcomes

Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	_____7_____
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	____7_____
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	____8_____
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	_____N/A_____
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	_____9_____
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	_____N/A_____
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	_____10_____
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	_____18_____

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	19
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	19
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	20
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	20
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	20
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	21
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	21
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	21
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	21

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	21
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	21
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	21
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	21
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	22
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	22
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	23
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	23
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	24
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	24
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	24
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	24
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.

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Effects of exercise intensity and nutrition advice on myocardial function in obese children and adolescents - a multi-centre randomized controlled trial study protocol

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Effects of exercise intensity and nutrition advice on myocardial function in obese children and adolescents - a multi-centre randomised controlled trial study protocol

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We reported this protocol in accordance with the SPIRIT (Standard Protocol Items: Recommendations for Interventional Trials) 2013 statement.

Keywords: Paediatric obesity, myocardial function, vascular function, visceral adipose tissue, high intensity interval training, nutrition & dietetics

Word count: 6,477

ABSTRACT

Introduction: The prevalence of paediatric obesity is increasing, and with it lifestyle-related diseases in children and adolescents. High intensity interval training (HIIT) has recently been explored as an alternate to traditional moderate intensity continuous training (MICT) in adults with chronic disease and has been shown to induce a rapid reversal of subclinical disease markers in obese children and adolescents. The primary aim of this study is to compare the effects of HIIT with MICT on myocardial function in obese children and adolescents.

Methods and analysis: Multi-centre randomised controlled trial of 100 obese children and adolescents in the cities of Trondheim (Norway) and Brisbane (Australia). The trial will examine the efficacy of HIIT to improve cardiometabolic outcomes in obese children and adolescents. Participants will be randomised to (1) HIIT and nutrition advice, (2) MICT and nutrition advice or (3) nutrition advice. Participants will partake in supervised exercise training and/or nutrition sessions for 3 months. Measurements for study endpoints will occur at baseline, 3 months (post-intervention) and 12 months (follow up). The primary endpoint is myocardial function (peak systolic tissue velocity). Secondary endpoints include vascular function (flow-mediated dilation assessment), quantity of visceral and subcutaneous adipose tissue, myocardial structure and function, body composition, cardiorespiratory fitness, autonomic function, blood biochemistry, physical activity and nutrition. Lean, healthy children and adolescents will complete measurements for all study endpoints at one time point for comparative cross-sectional analyses.

Ethics and dissemination: This randomised controlled trial will generate substantial information regarding the effects of exercise intensity on paediatric obesity, specifically the cardiometabolic health of this at-risk population. It is expected that communication of results will allow for the development of more effective evidence-based exercise prescription guidelines in this population while investigating the benefits of HIIT on subclinical markers of disease.

Trial Registration: NCT01991106

Strengths and limitations of this study

- To our knowledge, this multi-centre trial is the first to use a combined exercise and nutrition programme to examine the effect on myocardial function in obese children and adolescents. It also one of few trials to explore the efficacy and feasibility of high intensity interval training in this population.
- Strengths of this multi-centre trial lie in the rigor of the twelve-week exercise and nutrition intervention. The majority of exercise sessions will be supervised to ensure that the correct exercise intensity is achieved at all times.
- Extensive resting and exercise outcome measures will be performed at both trial centres. Several measures are sensitive to small but important longitudinal changes and are novel in paediatric obesity.
- Like any paediatric longitudinal trial, challenges remain around growth and maturation over the trial period, which have the potential to confound study endpoints. While the primary study endpoint is related to cardiac growth, any changes in cardiac size over the one-year program will be accounted for using normalisation methods.
- Maturational changes will also be accounted for through Tanner Stages of puberty however these may be subject to self-report error.

INTRODUCTION

Paediatric obesity rates have increased over the last two decades, and is now prevalent in 3-15% of the paediatric population according to the International Obesity Task Force definition of obesity [1]. More than 50% of obese children will become obese adults [2] with a significantly increased risk of developing non-communicable diseases including cardiovascular diseases, cancer and type 2 diabetes mellitus (T2DM) [3]. In 2012, these diseases accounted for 63% of deaths worldwide [3] and 77% of disease burden in Europe [4]. Indeed, a childhood and adolescent body mass index (BMI) above the 95th percentile is a strong predictor of adult mortality rates [5]. Furthermore, children and adolescents who had a baseline BMI between the 85th and 95th percentiles, defined as overweight, had a 30% increase in all cause mortality. This increased risk of death was independent of their adult BMI [5].

Obese children and adolescents may show abnormal myocardial function when assessed through resting and stress echocardiography [6-10]. Echocardiographic techniques such as tissue Doppler imaging are able to detect subclinical heart disease [11]. Previous investigations have shown significantly reduced Doppler tissue velocities in obese youth compared to lean, healthy age-matched control participants [12]. In particular, peak systolic tissue velocity (s') closely reflects left ventricular contractility [13], which can be improved with short-term exercise training [14].

Increased BMI or overweight in early life (1-9 years) is associated with coronary artery disease [15] and it is acknowledged that atherosclerotic processes begin in childhood [16]. Impaired vascular function determined by flow mediated dilation (FMD) of the brachial artery has been observed in a number of obese paediatric studies [17-22]. Visceral adipose tissue is increased in obesity which results in a greater release of bioactive mediators [23]. These influence the function of

adipose tissue and contribute to chronic disease, having a substantial impact on insulin sensitivity, inflammation and subsequent risk for dyslipidemia, T2DM and atherosclerosis [24].

Current pediatric guidelines for treating paediatric obesity recommend lifestyle modification to encourage family based behavior change leading to a reduction in energy intake and increase in physical activity [25,26]. The current treatment of pediatric obesity appears to have a low success rate, most likely due to the heterogeneous causes of obesity. Several recent meta-analyses and reviews have demonstrated that the effectiveness of pediatric obesity treatment is limited [27-29]. The response to treatment differs substantially between clinical centers and treatment success appears to be age-related. A large European registry study showed significant treatment effects following a variety of lifestyle interventions including exercise programs, nutrition education, psychological intervention and parental education, in less than 10% of participants over two years of follow-up [30]. Furthermore, the treatment was least effective in participants older than 12 years old. However, the low success rate was in part accounted for by a high dropout rate. Two recent reviews suggest that lifestyle and exercise interventions in obese children and adolescents can lead to improvements in anthropometric and cardiometabolic outcomes. These reviews are not inclusive of several important outcomes such as myocardial and vascular function, visceral adipose tissue or cardiorespiratory fitness [27,31]. Myocardial and vascular function outcomes have prognostic significance [32,33], are able to identify subclinical disease [7,34,35], and may be improved with exercise training [12,36]. There is growing evidence that paediatric obesity is associated with subclinical structural and functional cardiovascular alterations [12,35]. Myocardial dysfunction is more easily unmasked during stress as it precedes resting abnormalities

[12,37] Furthermore, low cardiorespiratory fitness has shown independent associations with all-cause mortality [38] and improvements in cardiorespiratory fitness may attenuate risk of metabolic disease [39] independent of visceral adipose tissue [40]. There is minimal evidence regarding the effect of exercise intensity on these novel markers in paediatric obesity.

Current worldwide data show that less than 50% of children and adolescents accumulate the minimum recommended 60 minutes of moderate to vigorous intensity physical activity every day [41-46]. Moreover, obese children spend approximately 100 minutes a day being less physically active than healthy weight or overweight children [47]. The promotion of high intensity exercise in this obese paediatric group may be an alternative to improve cardiometabolic outcomes. High intensity interval training (HIIT) has recently been explored as an alternate exercise to moderate continuous intensity training (MICT) in healthy adults as well as those with chronic disease. HIIT involves a short bout of exercise at a high intensity, interspersed by recovery periods in preparation for the next bout. HIIT has resulted in improved health markers in adults with cardiometabolic disease [48] while demonstrating time-efficiency [49]. Four studies to date have examined the physiological effects of HIIT in obese children and adolescents and reported positive findings [12,50-52]. Children and adolescents expressed increased enjoyment during high intensity interval training [49] and the stop-start nature of HIIT may reflect play-based activities traditionally observed during childhood. This is particularly important as enjoyment is a strong determinant of exercise adherence [53,54]. In fact, a 'lack of enjoyment' is frequently reported as a barrier by obese children [55].' While the chosen HIIT protocol (40 minutes; 10 minute warm up, 4 x 4 minute intervals interspersed by 3 minutes active recovery, 5 minute cool down) has a similar time commitment to the MICT protocol

(44 minutes), this is a result of equalising energy expenditure between training types [56]. We have previously shown that this particular HIIT protocol in obese adolescents can almost normalise cardiac function to that observed in lean counterparts and high compliance to the HIIT protocol was noted [12]. However the pilot trial had a small sample size and did not include comparative treatments [12]. The 4 x 4 HIIT protocol has shown great efficacy in clinical adult populations including patients with heart failure, coronary artery disease, hypertension, obesity and the metabolic syndrome [48]. Weston et al. (2014) recommend the use of this protocol due to greater time efficiency; i.e. increases in cardiorespiratory fitness following a HIIT program were nearly double the increases seen after a MICT program (19.4% versus 10.3%) [48]. In the studies reviewed, Weston et al. (2014) found that HIIT was able to elicit many superior benefits to MICT, albeit in only a slightly shorter time period [48]. This randomised controlled trial aims to examine the physiological efficacy of HIIT compared to MICT in a multi-centre randomised controlled trial, thereby improving the current literature and informing the treatment options for paediatric obesity.

OBJECTIVES

The primary aim of this randomised controlled trial is to compare the effectiveness of three 12-month interventions: HIIT and nutrition advice, MICT and nutrition advice, or nutrition advice alone on myocardial function in obese children and adolescents. It is hypothesised that HIIT will be superior in improving myocardial function compared to MICT. Secondary outcome measures will include vascular function, body composition (total, visceral and subcutaneous adipose tissue depots and lean muscle mass), cardiac structure, cardiorespiratory fitness, autonomic function, serum fasting lipids and insulin sensitivity, biomarkers of inflammation,

satiety and oxidative stress markers, physical activity levels and nutrition. Phase I of the trial will examine the effectiveness of an intensive 3-month period on the stated outcomes with assessments at baseline and after 3 months of supervised training. Phase II of the trial aims to determine the amount of supervision required to maintain exercise habits over a 9-month home-based training period. For this phase, participants will be re-randomised to 1) monthly supervised exercise or 2) home-based exercise only for the 9-month period that follows. Final assessments will be completed at 12 months. Lastly, the trial aims to determine whether any of the intervention arms are able to improve and normalise outcomes comparable to those found in healthy weight children and adolescents. It is for this comparative purpose that an age-matched, lean and healthy control group of children and adolescents will be assessed at a single time point.

METHODS AND ANALYSIS

Study setting

This is a multi-centre randomised controlled intervention trial. The study centre is located at The Norwegian University of Science and Technology (NTNU), Trondheim, Norway. The other centre is at The University of Queensland (UQ), Brisbane, Australia. Testing and training will take place in the university research laboratories at these institutions and hospital outpatient settings (St. Olav's Hospital in Trondheim & The Wesley Hospital in Brisbane).

Participants and eligibility criteria

The study cohort will include 100 obese and 100 lean, healthy control children or adolescents aged between 7-16 years old. Obesity in this population will be defined as a BMI equal to or greater than the 95th percentile (age and sex specific) [57]. Study exclusion criteria include hypertension (defined as blood pressure above the 95th

percentile for systolic or diastolic values), any history or evidence of heart disease and/or an abnormal resting or stress echocardiography which indicates it would be unsafe to participate, any chronic disease e.g. chronic asthma, kidney disease, diabetes, current smoking habits, or an orthopaedic/neurological disorder that may limit ability to exercise, diagnosed attention deficit hypersensitivity disorder and use of steroid medications. Conditions not specifically mentioned above may serve as criteria for exclusion at discretion of the clinical site. Furthermore, if medical conditions become apparent during testing or training in participants, medical advice will be sought and the intervention may be discontinued in the individual. Lean healthy control participants must have an age and sex specific BMI in the healthy range (5^{th} – 85^{th} percentile) to be included [57]. Study exclusion criteria are identical in lean healthy control participants. The lean healthy control participants will not partake in an intervention but will be assessed for cross-sectional comparative analyses.

Interventions

A randomised block design will be used. Obese participants will be randomised to one of three groups and will be stratified according age and sex. Lean participants will be stratified to the same groups but no intervention will be administered (Figure 1).

Exercise Protocol

The exercise intervention involves a combination of supervised and unsupervised exercise training sessions. Participants will attend at least two, up to three supervised training sessions each week for twelve weeks. If participants choose to attend two supervised sessions, they will be required to complete a third unsupervised exercise session at home. Following the three-month supervised period,

participants in each of the two exercise groups will be randomised to 'monthly supervised exercise' or 'home-based exercise only' from 3 to 12 months. During this time, participants in the HIIT and MICT groups will be asked to complete three unsupervised training sessions each week for nine months and the 'monthly supervised exercise' group will be asked to attend once-monthly supervised training sessions at the clinical site.

Supervised exercise training (HIIT and MICT) will consist of walking or running on a treadmill, or cycling on a stationary bike based on participant preference. During the unsupervised exercise session the mode can vary. Necessary adjustments to speed/grade, and resistance will be made over the course of the intervention to ensure that target heart rate zones are achieved at all times. Heart rate, rating of perceived exertion (Pictorial Children's Effort Rating Table - PCERT), and exercise mode will be recorded in a training booklet during supervised and unsupervised exercise sessions. In the event of limited access to a heart rate monitor during unsupervised sessions, participants will be asked to replicate the supervised session as closely as possible (i.e. identical treadmill speed/grade for intervals and active recovery periods) and to aim for similar RPE recordings as in the supervised sessions. Participants who wish to complete an unsupervised session each week will be provided with a separate booklet, which will be kept alongside the clinic version. Each participant will also receive a training booklet for the follow up period allowing them to record details of each session completed.

High intensity interval training

Participants randomised to HIIT will perform a 10-minute warm up at 60-70% of maximal heart rate (HRmax). Following this, they will walk, run or cycle at 85-95% of their maximal heart rate for four, 4-minute intervals, with 3 minutes of active

recovery (50-70% of HRmax) between the intervals. Participants will perform a 5-minute cool down period at the end amounting to a total exercise time of 40 minutes.

Moderate intensity continuous training

Participants randomised to the MICT group will walk, run or cycle continuously at 60-70% HRmax for 44 minutes to approximate the average energy expended by the HIIT group as previously calculated by our research group [56].

Nutrition advice

HIIT, MICT and nutrition groups will receive eight to ten, 20-minute individual nutrition sessions with a dietitian over the twelve-month period. Content of the sessions will include healthy food choices, portion sizes and regular meal times. The nutritional advice given will reflect current Norwegian and Australian eating guidelines and will be location specific [58,59]. The nutrition group will not be provided with any prescribed supervised exercise.

Outcomes

Measurements will take place at baseline, 3 months (end of the supervised exercise intervention period) and at 12 months follow up.

Participant preparation guidelines

Participants will be informed of preparation requirements, which will be checked prior to the assessments.

Stress echocardiography & cardiorespiratory fitness testing

Assessment of myocardial function and structure, and maximal oxygen uptake will require participants to refrain from eating heavy meals up to two hours before the testing session and avoid any foods containing caffeine for this time period.

Vascular function, body composition and blood collection

Assessment of vascular function, body composition (BodPod) and blood collection will be completed in a fasted state (8-12 hours) and participants will be instructed to avoid caffeine, vitamin C, alcohol, drugs, stimulants and medications for this time period. Additionally, participants must refrain from intense exercise for 48 hours prior to testing. To avoid dehydration, participants will be instructed to drink at least 0.5L of water before attending the assessment.

Myocardial function (rest and stress echocardiography)

Primary Outcome Measure: Peak systolic tissue velocity (S') at rest

Secondary Outcome Measures: S' (during exercise, both ventricles), S' (rest, right ventricle), peak diastolic tissue velocities (both ventricles), tricuspid annular plane systolic excursion (TAPSE), global strain and strain rate, aortic flow, cardiac dimensions.

A full resting echocardiogram will be performed with a Vivid 7/E9 ultrasound machine (GE Vingmed Ultrasound, Horten, Norway) using a phased-array transducer (GE M3S). Three cine loops from the three standard apical planes (four-chamber, two-chamber and long-axis) and the right ventricle will be recorded in grey scale harmonic mode and tissue Doppler mode simultaneously. LV standard Doppler echocardiographic indices will be measured and body surface area (m^2) will be used to normalize cardiac dimensions for differences in body size. Mitral annulus excursion, pulsed wave tissue Doppler velocities; peak systolic (S'), peak early diastolic (e') and peak late diastolic (a') will be measured at the AV-plane level in 4-chamber and 2-chamber view and a mean of the 4 points will be used. Right ventricle function standard Doppler echocardiographic indices will be measured and TAPSE, S', e', a'. Deformation (strain) and deformation rate (strain rate) will also be analysed by speckle tracking (2D strain) and tissue Doppler imaging.

Following the resting echocardiogram, individuals will exercise on a stationary cycle ergometer in an upright position. The exercise protocol will start at an intensity of 25W with 25W increments every three minutes until participants have attained their maximum heart rate or are no longer able to maintain a constant cadence. Recordings will be made at baseline and peak assessing apical 4-chamber and 2-chamber in B-mode and tissue Doppler as well as mitral and aortic flow. A three lead electrocardiogram (ECG), blood pressure and ratings of perceived exertion (PCERT) will be monitored and recorded at the end of each stage.

EchoPAC™(GE Vingmed Ultrasound AS, Horten, Norway) will be used for all echocardiographic analysis by an investigator blinded to the group allocation of the subjects.

Visceral, subcutaneous and total abdominal adipose tissue (MRI)

At the Brisbane site, visceral, subcutaneous and total abdominal adipose tissue adipose tissue will be measured using a 1.5 Tesla magnetic resonance imaging (MRI) system (Siemens Symphony Sonata, Siemens, Erlangen, Germany) equipped with a 6 channel body matrix coil and a 6 channel spine coil. Subjects will be positioned supine inside the magnet and transversal images will be acquired using TRUFISP (true fast imaging steady state precision) technique with breath-hold (repetition time = 3.76 ms; echo time = 1.88 ms; flip angle 75deg; matrix = 220 x 256; rectangular field of view (FOV) = 400 mm x 400 mm; slice thickness = 8mm; 14 slices; acquisition time = 12 sec). We will acquire 14 axial slices, 8mm thick centred over the umbilicus during breath-hold using a Dixon technique.

At the Trondheim site, visceral, subcutaneous and total abdominal adipose tissue will be measured using a 3 Tesla MRI system (Siemens Skyra; Siemens, Munich, Germany) equipped with an 18 channel body coil and a 32 channel spine

coil. Subjects will be positioned supine inside the magnet and transversal images will be acquired using T1-weighted Dixon vibe sequences with breath-hold (repetition time=4,04ms; echo time=1.3ms and 2.50ms; flip angle=9deg; matrix=320 x 256; rectangular field of view (FOV)=380mm x 309; Slice thickness=3mm; 52 slices; Number of averages=1; Band width=1120Hz/pixel; GRAPPA parallel imaging acceleration factor 2, acquisition time=17 sec). If the given FOV is too small to cover the whole subject, the FOV is increased sufficiently before scanning. The Dixon sequences will be acquired twice to assure a set of successful images.

The MRI scans will be exported and anonymised. Images will be converted to NIfTI format and analyzed using in-house software developed in MATLAB (TheMathWorks, Inc, Natick, MA). Five consecutive slices at and above the L4/L5 vertebral disc, with localizing images used to confirm the level, will be selected for quantification of visceral and subcutaneous fat in each participant. Two regions of interest (ROIs) will be manually drawn on the images by a trained radiologist. One ROI will delineate the subcutaneous compartment and the second ROI will delineate the intra-abdominal and retroperitoneal areas together to quantify visceral adipose tissue. Visceral and subcutaneous adipose tissue will be summed to quantify total adipose tissue. The area of the fat inside each ROI will be calculated by counting the number of pixels with intensities above a selected threshold and multiplied by the pixel area. Slices will be manually adjusted for threshold intensity in order to compensate for lack of uniformity between slices. Values calculated from the five slices will be averaged to provide a mean area (cm^2).

Vascular function (flow mediated dilation)

Endothelial function of the brachial artery will be measured via flow mediated dilation using high-resolution vascular ultrasound (12-14 MHz ultrasound- Doppler

probe, Vivid 7 system/Vivid I system; GE Vingmed Ultrasound AS, Horten, Norway) according to current guidelines [60]. Participants will lie supine for ten minutes in a quiet, dark environment prior to commencement of the procedure. A transducer will be placed against the brachial artery and following image optimisation, baseline images will be recorded for at least 30 seconds in live duplex mode (simultaneous B-mode diameter and pulsed-wave Doppler velocity signals). A blood pressure cuff will then be placed distal to the region of interest and inflated for five minutes at 200mmHg. This will reduce blood flow to the hand. Upon cuff release, 10 beat cine-loops will be recorded in duplex mode (B-mode diameter and pulsed-wave Doppler velocity signals) at 10s, 30s, 60s, 90s, 120s, 150s and 180s (UQ), or continuously for 3 minutes (NTNU) to measure the change in the diameter of the artery following increased blood flow and shear stress to the vascular wall. Data will be assessed by custom-made automated edge-detection software, which is independent of investigator bias. Brachial artery diameter and shear rate will be quantified from the ultrasound studies to assess flow-mediated dilation.

Cardiorespiratory fitness

Maximal oxygen uptake (VO_2max) will be measured during uphill treadmill walking or running using expired air gas analysis (Metamax 3B, Cortex Biophysik, GmbH, Leipzig, Germany or Jaeger Oxycon Pro, CareFusion, Hoechberg, Germany). An incremental, ramp protocol will be used for all participants. A four minute warm up at 4km/h and 0% grade will precede the test however the speed can be modified to match the preferred walking speed of the participant. A walking or running protocol is available depending on participant preference and fitness.

The walking protocol consists of one minute stages where speed is set at preferred walking speed and gradient is increased by 2% each minute. If the

participant reaches a steep gradient (this will be adjusted by researchers depending on age and height of participant: <12yrs ~12%, ≥12 yrs ~16%), speed will be increased by 2km/h each minute thereafter.

The running protocol consists of one-minute stages where gradient is set at approximately 10% (this will be adjusted by investigators depending on age and height of participant) and speed is increased by 1km/h each minute. If the participant reaches a speed that can no longer be safely increased, gradient will be increased by 1% each minute thereafter.

A levelling off of oxygen uptake (VO_2) despite increased workload and respiratory exchange ratio (RER) ≥1.05 will be used as criteria for VO_2max.
A levelling off (plateau) in VO_2 will be defined using 30-second epochs. If the VO_2 increase is $\frac{<150 ml O_2/min}{Body\ mass}$ with an increase in workload, then a plateau is assumed. If the participant has reached a plateau and the RER criterion has been satisfied then the two highest consecutive 30-second values will be averaged to obtain the VO_2max value. If the participant has not reached a plateau, VO_2peak is determined by using the average of the two highest values attained. It is highly likely that most participants will not reach a VO_2max and therefore group values will be reported as VO_2peak.

Heart rate will be measured continuously during the test (Polar, Polar Electro, Kempele, Finland), to define HRmax.

Heart rate variability

Participants will lie supine for ten minutes in a quiet, dark environment prior to commencement of the procedure. Participants will be asked to lie as still as possible for five minutes while an ECG trace is monitored and recorded for calculation of heart rate variability. RR intervals obtained from the ECG will be processed using Kubios HRV (University of Eastern Finland, Finland).

Body composition

At the Brisbane site, dual energy x-ray absorptiometry (DXA) will be used to determine body composition (adipose tissue and lean muscle mass). This will require the participant to lie motionless while an x-ray of their entire body is taken using the DXA scanner (Hologic, QDR Series, MA, USA). The duration of a whole body scan is 7 minutes.

At the Trondheim site, a BodPod (COSMED, Rome, Italy) will be used to determine body composition (adipose tissue and lean muscle mass). Participants are required to be fasted (8 hours) and will be tested in minimal clothing (underwear only). The procedure takes 15 minutes in total with 5 minutes spent in the BodPod.

Blood biochemistry

Venous blood samples will be collected from a superficial antecubital vein according to standard phlebotomy procedures. Samples will be collected into three vacutainers containing EDTA, fluoride oxalate and clot activators. Vacutainers will be stored on ice, or left to clot at room temperature for at least 30 minutes (serum samples). Two aliquots of whole blood will be pipetted out and following this, samples will be centrifuged at 2500 rpm, 4 degrees Celsius for 10 minutes. Plasma/serum will be aliquotted and stored at -80 degrees Celsius for later analysis. Samples will be analysed for lipids (total cholesterol, LDL, HDL, triglycerides), glucose and insulin (to establish insulin resistance and beta cell function using HOMA-IR) and CRP using spectrophotometry (Cobas Mira, Roche Diagnostics, Australia). Insulin resistance and beta cell function will be calculated using the HOMA model (based on fasting glucose and insulin concentrations) where $HOMA-IR = \frac{Glucose\ x\ Insulin}{22.5}$ and $HOMA-\beta = \frac{20\ x\ Insulin}{Glucose-3.5}$ %. Satiety hormones (ghrelin, leptin, peptide YY, obestatin), inflammatory markers (TNFα, IL-6, IL-10, PAI-1),

adiponectin and si-CAM-1 will be measured using specific ELISA kits (R&D systems, Inc., Minneapolis, MN, USA). Total nitrite concentration will be quantified using a commercially available assay for nitric oxide (NO_2-) detection (R&D systems, Inc., Minneapolis, MN, USA). Oxidative stress and antioxidant status will be measured using the following methodologies. Total F-2 isoprostanes will be extracted and analysed using a method developed by the Brisbane laboratory [61]. The laboratory coefficient of variation for this assay is 4.5%. Protein carbonyls will be analysed using adapted methodology from Levine et al. (1990) [62]. The laboratory coefficient of variation for this assay is 11.9%. Plasma glutathione peroxidase (GPx) activity will be measured spectrophotometrically (Cobas Mira, Roche Diagnostics, Switzerland) via the oxidation of NADPH to NADP by modifying methods [63,64]. The laboratory coefficient of variation for this assay is 2.4%.

Physical activity and nutrition measurements

Accelerometry will be used to measure physical activity at baseline and at the three-month assessment. Participants will be asked to wear an accelerometer for seven days (Brisbane: ActiGraph, Florida, USA and Trondheim: SenseWear, BodyMedia, Inc., Pittsburgh, USA). To be included in the analysis, participants will be required to have a minimum of four valid days, one of which must be a weekend day. Participants in Brisbane will be asked to wear the ActiGraph monitor during waking hours, except for when sleeping or during water-based activities. Participants will also be asked to keep a brief log to record wake/sleep times and any time the monitor was removed for >10 minutes (e.g. sleep, shower etc.). The ActiGraph accelerometer will be initialised to sample at 30Hz and data will be aggregated in 15-second epochs. To be considered a valid day, there must be a minimum of 10 hours of wear time and non-wear time criteria will be 60 minutes or more of consecutive zeros. Accelerometer cut points

previously validated in a paediatric population [65] will be used to determine average time per day spent in light physical activity, moderate physical activity and vigorous physical activity. ActiGraph data will be analysed using ActiLife software (version 6, Florida, USA). Participants in Trondheim will be asked to wear the SenseWear armband for 24 hours each day. The device will be removed during water-based activities. To be considered a valid day, at least 85% of a 24-hour day must be recorded and time spent in light physical activity (1.6-2.9 METS), moderate physical activity (3-5.9 METS) and vigorous physical activity (>6 METS) will be determined. SenseWear data will be analysed using BodyMedia (version 8.1, Pittsburgh, USA).

Participants will also complete a physical activity questionnaire designed by the Norwegian Directorate of Health with content focusing on total physical activity, physical activity at school and home, and weekday/weekend screen time [66].

Food record booklets will be given to participants and a three to four-day record will be requested (must include one weekend day). Food records will be analysed using either FoodWorks (Xyris Software, Australia) for the Australian cohort or using the food database KBS AE-07 and KBS software system (KBS, version 4.9, 2008, Department of Nutrition, University of Oslo, Norway) for participants in Norway.

Other measures

Height, weight, waist (WC) and hip circumferences (HC) and blood pressure will be measured using standard approaches [67].

Participant timeline

Figure 2 illustrates the schedule for measurement of outcomes and the intervention.

Sample size

The sample size was calculated for a one-way ANOVA analysis comparing the mean change in resting S' between the three groups from pre to post intervention. Although the data will be analysed using a linear mixed model (LMM), a simplified calculation of sample size for a one-way ANOVA provides a conservative estimate of the sample size required for the LMM. Simulations run by our research group support this assumption. For calculation of sample size, the clinically meaningful difference in means was set to 1cm/s, using the values 9.5, 10 and 10.5 cm/s for the nutrition, MICT and HIIT groups, respectively. The standard deviation, assumed to be equal for all groups, was set to SD=0.9 cm/s [12]. To obtain a power of 0.80 for the overall test of differences in means, using a significance level of 0.05, 17 individuals are required in each group. A further 40 individuals are required to account for the four stratification groups (Figure 1) included in all statistical analyses. To account for 15% dropout, a total of 105 individuals are required to enter the intervention. In order for the lean control group to be closely matched in age, sex and sample size, 105 lean participants are required for assessment at a single time point.

Recruitment

A variety of recruitment strategies will be employed at each site to achieve adequate participant enrolment and reach target sample size. At the Trondheim site a regular advertisement will be placed in the local newspaper. In addition, advertisements will published on the university and hospital websites and flyers will be distributed at strategic locations. Every six months, local health nurses and medical centres will be informed about the study. A website will be set up with a linked preliminary screening tool and program contact form. A video blog about childhood obesity research will be used for media outlets and publicity through newspapers and

television. Social media such as Facebook will also be used. Similar strategies will be used at the Brisbane site. An advertisement for the study will be placed in the university staff newsletter and flyers will be placed around the campus. Schools in a 30km radius will be emailed with a newsletter advertisement. Health & fitness centres and medical centres within a 15km radius will be asked to advertise the program on a noticeboard. A website will be set up with a linked preliminary screening tool and program contact form. Google AdWords will be employed as a continuous recruitment pathway strategy with approximately 10-15 clicks resulting in website views expected each day. Local dietitians and paediatricians will also be contacted for referrals into the program. Finally, media outlets will be contacted for newspaper and television coverage.

Assignment of interventions

Allocation sequence generation

Computer generated random numbers will be used for allocation sequence generation with stratification completed according to age and sex (see Figure 2.0). The four stratification groups will be:

1) Females 7-10 years
2) Males 7-12 years
3) Females 10-16 years
4) Males 12-16 years

Allocation concealment will be implemented using central randomisation through a web-based program that will generate the allocation sequence. A study investigator at each centre will enter the details of eligible obese participants and the web-based program will provide the group allocation for the intervention. The investigator will then inform the parent or guardian. Lean and healthy control participants will also be registered and stratified using the web-based program.

Blinding

As this is an exercise intervention, trial participants cannot be blinded to group assignment. However, outcome assessors, and data analysts will be blinded to intervention assignment. Outcome assessors of the primary outcome are independent of the clinical centre and will not interact with participants outside of the assessments. Participants will be asked not to divulge their group allocation during testing visits. All data is stored using participant ID number only and data analysis that is subject to investigator bias will occur without knowledge of intervention assignment.

Data management and analysis

Data management

Double data entry will be administered at each site to ensure data quality. Data will be stored in a re-identifiable format (participant numbers only). A password-protected sheet will enable the participants' numbers be linked to names when required.

During and after the research project, data on paper will be kept in a locked filing cabinet. Electronic data will be stored on password-protected computers or external hard drives with access granted only to members of the research team. Tissue samples will be identified by participant number only and will be stored in a secure locked area.

Information collected will be disposed of ten years after study completion. Paper documents will be shredded and disposed of while electronic information will be erased.

Statistical methods

An intention to treat analysis will be used. A per protocol analysis will also be conducted where completion of 80% of the 3-month intervention is required. Data

will be analysed with SPSS Statistics (IBM, NY, USA), Stata (TX, USA) and R (R Core Team, Vienna, Austria). Normality of data will be checked using one or more normality tests. Descriptive statistics will be computed for variables of interest and continuous data will be reported as means and standard deviations if data is normally distributed. Non-continuous and non-normally distributed data will be reported as frequencies, medians and interquartile ranges.

Statistical analysis comparing between group differences (3 groups) following the intervention will be conducted using a linear mixed model (LMM). This technique calculates between-group and within-in group differences (from baseline to post-intervention) within the same model. A linear mixed model is also able to adjust for stratification variables (age, sex) and for a centre effect if present. Adjustment for a centre effect is particularly important, as there are minor discrepancies in the intervention and outcome methodologies, as well as a large climatic difference due to varying geographical locations of the clinical centres. In order to examine the effect of supervision during the nine-month follow up period (from three to 12 months), an LMM will be used again with supervision included as an additional explanatory variable in the model.

To compare between-group differences in binary categorical data following the 3-month intervention, a generalised linear mixed model (GLMM), which accounts for time correlations, will be used.

Monitoring

Harms

If a participant develops a medical or surgical illness during the study, the Data Monitoring Committee in cooperation with the participant's general practitioner will ascertain continuing or resuming participation in the intervention. In the event of

a medical emergency occurring at the clinical sites, the study staff will undertake, under direction of the principal investigator or designated staff, all necessary supportive medical care.

All adverse events (AE) will be reported between the first trial-related study procedure and the last during study intervention. Medical events that occur between the signing of the Informed Consent Form and the final study-related procedure will be documented in the medical history.

Participants should voluntarily report any AE's or in response to general, non-directed questioning (e.g., "How has your health been since the last visit?"). For each AE volunteered by the participant, the investigator will obtain all the information required to complete the AE documentation. All AE's regardless of seriousness, severity or presumed relationship to the study will be recorded using medical terminology in the source document and on the case report form. Safety related events will be reported in a timely fashion as required by the Data Monitoring Committee and the Ethics Committees responsible for the study.

ETHICS AND DISSEMINATION

Research ethics approval

The investigation protocol and procedures have been approved at each clinical site. At NTNU, Norway, the Regional Committee for Medical and Health Research Ethics has approved this project (reference number 2009/1313-4). At UQ, Australia, The University of Queensland Human Research Ethics Committee (reference number 2013000539), The Mater Hospital Human Research Ethics Committee (reference number HREC/13/MHS/119/AM01) and Uniting Care Health Human Research Ethics Committee (reference number 1324) has approved this project.

Consent and assent

Participants and their parents will provide informed consent after the principal investigator has briefed them on the study and answered questions. Two separate informed consent sheets will be signed with content adjusted to suit a paediatric population (participant and parent/guardian signs one while the other is signed by the participant only).

Confidentiality

Information collected directly from participants will be in a re-identifiable form and any information collected for, used in, or generated by this project will not be used for any other purpose. The site principal investigator and associated research personnel such as the study dietitian will have access to information.

Dissemination policy

Results of this clinical trial aim to be disseminated through peer-reviewed journal articles, conference abstracts and presentations, as well as media publications. It is hoped that results of the clinical trial will inform future paediatric obesity management strategies.

Patient and public involvement (PPI)

PPI strategies were implemented following the conclusion of preceding pilot trial. Several organisations were involved in the conception of protocol design in Brisbane (Child Obesity Program at the Mater Children's Hospital/ Lady Cilento Children's Hospital and The Children's Nutrition Research) and Norway (Centre for Obese Adults and Children at St. Olav's Hospital, the Cardiac Exercise Research Group at NTNU, the Norwegian Physiotherapist Association, as well as local schools and PE teachers in Trondheim). Six clinicians were also actively involved during the protocol development phase including cardiologists, paediatric endocrinologists, and

dietitians. The conclusion of the pilot trial provided an opportunity to receive feedback from the patient population in question. While a proportion of obese adolescents disliked HIIT until they improved their cardiorespiratory fitness, the majority reported enjoyment, which resulted in high training attendance and compliance. Freedom with exercise modality also increased HIIT feasibility in this group. A similar protocol was therefore implemented in the current intervention. During the ongoing clinical trial, feedback from participants and families has been welcomed and resulted in minor protocol adjustments. Notable adjustments include the structure of assessments and frequency of supervised exercise sessions in order to aid adherence.

DISCUSSION

Reduced physical activity and poor nutrition are the main causes of obesity in children and adolescents. A large proportion of the paediatric population does not meet exercise guidelines and energy intake in obese children is often larger than in healthy weight children [68,69]. Consequently, paediatric obesity has increased steadily in several countries over the last thirty years [70-72]. Current worldwide data suggest that only 5-50% of children and adolescents are meeting current exercise guidelines [41-46,73]. Furthermore, obese children are less physically active compared to healthy weight children [74,75] and spend more time in sedentary activities [76,77]. Low levels of leisure time physical activity are associated with an increased risk of cardiovascular and metabolic diseases [78]. Therefore, encouraging all children to increase their levels of physical activity and reduce their sitting time could help to avoid excess weight gain and associated health risks [79]. We therefore are striving to investigate a time efficient form of exercise, which could potentially induce physiological changes and reduce cardiovascular risk factors in obese children

and adolescents. The importance of nutrition in this population stipulated that the exercise interventions were combined with nutrition advice, and compared to a nutrition advice only group as well.

This clinical trial involves the largest known cohort of obese children and adolescents in a HIIT intervention to date. Valuable information about the effects of exercise intensity on cardiac and vascular structure and function, body composition, cardiorespiratory fitness, biochemistry markers, physical activity, nutrition and quality of life in obese children and adolescents will be gathered. Importantly, this trial will contribute to the currently small, but important, body of evidence exploring cardiac function during exercise. While resting function may remain normal in paediatric obesity, stress echocardiography may unmask subclinical cardiac disease [35] and lead to better patient management and outcomes.

Dissemination of the knowledge gained from this trial is expected to inform physical activity and exercise guidelines for this specific population in an attempt to dampen the impact that obesity has on physiological systems as well as reduce risk factors for future development of comorbidities such as T2DM and heart disease.

Contributorship statement: Each author: (1) made substantial contributions to conception and design; (2) assisted in drafting the article and provided important intellectual content and (3) gave final approval of the version to be published. Given the multi-disciplinary nature of this trial, specific contributions were made by each author. CI and AT conceptualised the trial and drafted the original study methods. KD, JC and CI refined study methods and will be the project managers of this multi-centre research trial. CI and PC expertise lie in assessment of myocardial function and structure while DJ and MSH specifically assisted with non-invasive methodologies for assessing vascular health. MH and EH designed the protocol for assessment of visceral and subcutaneous fat, while SK, MH and EH developed analysis methodologies for this outcome. PD and SH expertise lie in paediatric nutrition and therefore contributed to design of the nutrition intervention. GL has extensive experience in paediatric endocrinology, and paediatric obesity lifestyle interventions. GL assisted with general study design and identifying blood biochemistry variables of interest. SJ assisted with selecting an activity monitor device and with study design to collect and analyse physical activity levels. AT and KD designed the methods for the maximal oxygen consumption test. TR specifically contributed to biostatistics of the trial including sample size and power calculations, and statistical analysis methods.

Competing interests statement: Dr. Coombes reports grants from Coca Cola, personal fees from Tolmar Pharmaceuticals, personal fees from Novo Nordisk Pharmaceuticals, outside the submitted work. The remaining authors have read and understood BMJ policy on declaration of interests and declare that they have no competing interests.

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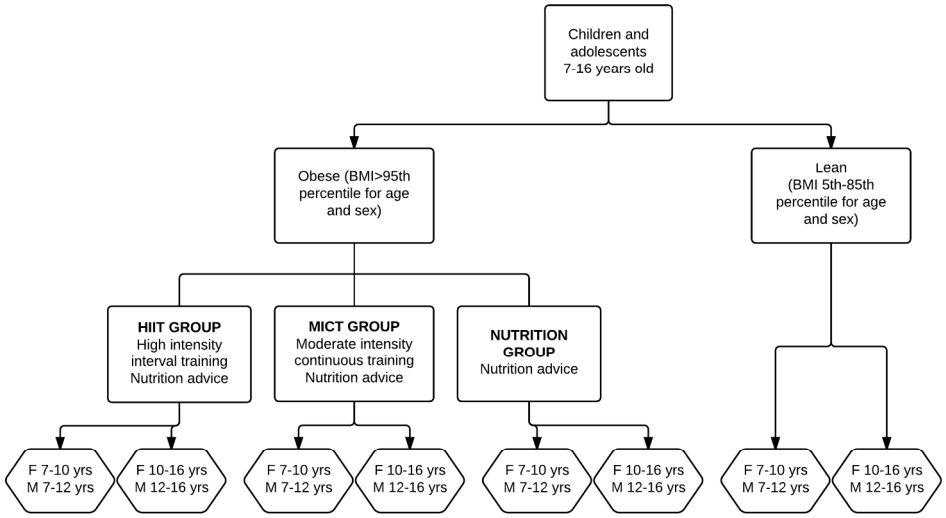

Figure 1: Intervention groups/ Stratification
200x114mm (300 x 300 DPI)

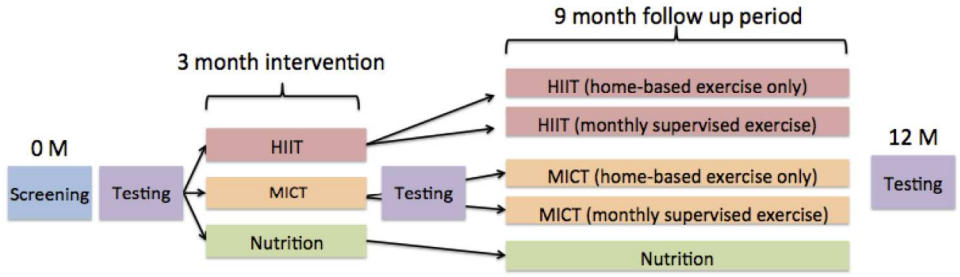

Figure 2. A schematic illustrating a time schedule for enrolment, intervention and assessment of obese participants.
297x209mm (300 x 300 DPI)

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item No	Description	Addressed on page number
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	1
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	2
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	26
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1
	5b	Name and contact information for the trial sponsor	1
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	N/A
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A

Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	4
	6b	Explanation for choice of comparators	5
Objectives	7	Specific objectives or hypotheses	6
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	7
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	7
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	8
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	N/A
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	9
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	N/A
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	10
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	18

Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	19
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	19
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	20
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	20
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	20
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	21
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	21
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	21
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	21

Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	21
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	21
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	21
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	21
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	22
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	N/A
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	22
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	N/A
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	23
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	23
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	N/A
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	24
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	24
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	24
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	24
	31b	Authorship eligibility guidelines and any intended use of professional writers	N/A
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "Attribution-NonCommercial-NoDerivs 3.0 Unported" license.