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The Effects of a Jump Rope Exercise Program on Vascular Health, Inflammatory Markers in Prehypertensive Adolescent Girls: full study protocol and statistical analysis plan

NCT#03534427

Study protocol

Participants

Forty girls (age 14-16) volunteered to participate in this research study. All participants were classified as prehypertensive (120-140 mmHg systolic BP and/or 80-90 mmHg diastolic BP) and had abdominal obesity (waist > 80 cm, BMI equal to or greater than the 95th percentile for age and sex). All participants were considered to be inactive, meaning they participated in less than 1 hour of regular physical activity per week within the last 6 months. Other exclusion criteria included a previous musculoskeletal injury within the last year that may negatively affect participation, as well as cardiovascular, pulmonary, adrenal, pituitary, and thyroid diseases. Participants who were already involved in a regular exercise program, on daily medication(s), including antioxidants and fat loss supplements, or weight loss diet plans were also excluded from participation. All procedures performed in this study were in accordance with the ethical standards of the institutional research committee at Pusan National University (PNU IRB/2016_105_HR) and with the tenets of the Declaration of Helsinki. This study was registered in Clinicaltrials.gov (NCT03534427). Informed consent was obtained from all individual participants and their parent/guardian(s) included in this study. Participant characteristics can be seen in Table 1.

Study design

A two-armed, parallel experimental design was used. The jump rope exercise intervention took place during the months of March – June during the school year. Blood samples, anthropometrics, and vascular function parameters were assessed before (baseline) and after the 12-week jump rope exercise program at 8:00 AM (±1 hour) following an overnight fast. After baseline measurements were performed, the participants were randomly assigned to either the jump rope exercise group (EX, n = 20) or the control group (CON, n = 20). The participants in the EX group participated in an exercise program (50 minutes per day, 5 times per week for 12 weeks) that consisted of jump rope variations. Exercise was performed at 2:00PM (±1 hour) Monday-Friday. The EX group did not perform any additional exercise outside of this prescribed program during the 12 weeks. The participants in the CON group did not participate in the exercise intervention, or any outside exercise during the 12-week period, but were present and supervised in the laboratory at the same frequency, duration, and time of day as the EX group throughout the entire study period. Both groups of participants were advised weekly not to change their diet habits (~1920 kcal/day) for the duration of the study, and diet logs were obtained every week to keep track of dietary intake. Water intake was not recorded, however, participants were encouraged to consume approximately 2.5 liters of water per day (Sawka et

al. 2005). All exercise sessions were supervised by trained professionals and all measurements were taken by qualified researchers.

Blood sampling and analysis

Blood samples were collected at 8:00 AM (±1 hour) after an overnight fast at baseline and after 12 weeks from an antecubital vein. Each sample was centrifuged for 15 minutes at 3,000 rpm stored at -80°C for later analysis. Using the provided reagents, each blood sample was run in duplicate in accordance with the manufacturer's instructions for total nitrate and nitrite levels, which were analyzed using a Griess assay kit from Cayman Chemical (Ann Arbor, MI, USA) (Giovannoni et al. 1997). The amount of nitrate and nitrite produced in the reaction mixture was determined spectrophotometrically at 540 nm (OD540) using a microplate reader (Edwards et al. 2004; Masaki and Yanagisawa 1992). Levels of endothelin-1 (ET-1) in blood samples were measured with an enzyme immunoassay kit (Endothelin-1 Enzyme Immunoassay Kit, Cayman Chemical, Ann Arbor, MI, USA). The detection range for ET-1 using this assay was from ≥ 1.5 μmol/mL (Hodeib et al. 2010). C-reactive protein (CRP) was assessed using an enzyme immunoassay kit (C-Reactive Protein Enzyme Immunoassay Kit, Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer's instructions (Druwe et al. 2012).

Pubertal Development

Pubertal development was assessed using Tanner staging (breast and pubic hair stage) by self-reporting at the beginning of the study. Standard line drawings and written descriptions were provided, and participants selected the picture that most accurately reflected their appearance (Morris and Udry 1980).

Anthropometrics

Anthropometric measurements were taken at baseline and after 12 weeks. Height was measured without shoes using a stadiometer to the nearest 1.0 cm. Body composition was measured using bioelectrical impedance analysis (BIA) (InBody 230, Biospace, Seoul, Korea), which simultaneously recorded total body mass (nearest 0.1kg), percent body fat (nearest 0.1%), and fat-free mass (nearest 0.1 kg) (Seo et al. 2012). The InBody 230 specifically has been validated to be consistent with the doubly labeled water method (Beato et al. 2018) and dual x-ray absorptiometry for measurements of total fat mass, total lean mass, and percent body fat (Karelis et al. 2013). For BIA, the intra-assay coefficient of variation (repeated measures within individual) was 0.8 ± 0.9% and the inter-assay coefficient of variation (repeated measures between different days) was 0.3 ± 0.2%. BMI was calculated as body mass divided by the square of height (kg/m^2). Waist circumference was recorded using a standard tape measure to the nearest 0.1 cm

at the midpoint between the iliac crest and the lower rib. These values were used to determine adiposity in absolute terms; however, there are no standard values that are considered to represent high adiposity in children and adolescents (Flegal et al. 2010).

Vascular function

Resting systolic and diastolic BP (mmHg) and resting heart rate (bpm) were both measured in duplicate using an automatic sphygmomanometer (HEM-7113 INT; Omron Corp., Kyoto, Japan) and radial artery palpation, respectively, at baseline and after 12 weeks. The measurements were recorded after participants were in a seated position for 5 minutes. The average of the two measurements was recorded as the resting BP and resting heart rate. Brachial-to-ankle pulse wave velocity (baPWV, m/s), an indicator of peripheral arterial stiffness, was measured using applanation tonometry (SphygmoCor CPV system, AtCor Medical Ltd, Sydney, Australia), followed by data analysis (version 8.0, SphygmoCor Cardiovascular Management Suite, AtCor Medical Ltd, Sydney, Australia). Two measurements were collected, one at each time point, and averaged as previously described (Weber et al. 2004; Yambe et al. 2007).

Jump rope exercise program

Participants in the EX group participated in a jump rope exercise program for 12 weeks, 5 days per week, for 50 minutes per day (Table 2). This program was divided into a warm-up (5 minutes), the main exercise session (40 minutes of rope jumping variations), and a cool-down (5 minutes). Both the warm-up and the cool-down consisted of stretching, walking, and jogging. There were 7 main rope jumping exercises, including 1 line 2 jump, jumping feet together, running jumping, open side jump, open back and forth jump, and rock paper scissor jump. The jump rope exercise program intensity incrementally increased every 4 weeks: weeks 1-4 were at 40-50% HRR and 11-12 RPE, weeks 5-8 were at 50-60% HRR and 13-14 RPE, and weeks 9-12 were at 60-70% HRR and 15-16 RPE. These exercise intensities, frequencies, and durations were chosen to reflect previous studies that reduced fat mass, BP, and arterial stiffness in various populations (Arikawa et al. 2011; Beck et al. 2013; Ewart et al. 1998). The exercise intensity of the jump rope program was monitored using heart rate reserve (HRR) and Borg's rating of perceived exertion scale (RPE) of 6-20. Participant heart rate was monitored using a wearable Polar heart monitor (Electro, Oy, Kempele, Finland) during all exercise sessions in order to sustain the proper training intensity. There were 5 trainers supervising each exercise session, each specifically assigned to 4 participants. Heart rate monitors and RPE

were checked every 5 minutes during the 40-minute training session. If heart rate or RPE were either too low or too high, participants were encouraged to either increase or decrease their effort during the session.

Statistical analysis plan

The Shapiro-Wilk test was used to determine the normality of the data. Independent t-tests were used to determine baseline differences between the two groups. A two-way analysis of variance (ANOVA) with repeated measures [group (CON and EX) x time (before and after 12 weeks)] was used to compare the difference of changes between pre- and post-jump rope exercise program within and between groups on the dependent variables. Bonferroni correction was used to determine the effects of the jump rope training program over time. When a significant main effect or interaction was noted, paired t-tests were used for post-hoc comparisons. An effect size analysis was performed using eta-squared (η^2) for the two-way ANOVA, and interpreted 0.01, 0.06, and 0.14 as small, medium and large, respectively (Lee et al. 2018). Cohen's d was used for the t-tests, interpreted as 0.20, 0.50, and 0.80 as small, medium and large, respectively (Lee et al. 2018). All analyses were performed using SPSS 25.0 (SPSS Inc., Chicago, IL, USA). Data are presented as Mean ± SD. Statistical significance was set at $P \leq 0.05$. It was estimated that 40 participants would enable 80% power to detect a 5% decrease in baPWV after the jump rope exercise program based on previous literature (Figueroa et al. 2011).