Short-term effects of gene-physical activity interaction on obesity and related metabolic indicators in children: a randomized controlled trial

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Research design

Ethics statement

This study was approved by the ethics committee of the China Medical University (Ethics Approval No. [2020] 075). Every participant signed an informed consent form. All methods were performed in accordance with the Declaration of Helsinki and relevant guidelines.

Study Design and Participants

This study is based on a randomized controlled trial of a 4-week MVPA intervention conducted among children aged 10-12 years with overweight or obesity problems. We followed the established criteria of overweight and obesity in Chinese children and adolescents proposed by the Working Group on Obesity in China (WGOC) [14]. Children with physical activity-limiting diseases and a lack of baseline or post-intervention physical examination data were excluded. Then a total of 139 children with overweight or obesity were randomly allocated (2:1) to MVPA intervention group (n=93) or a control group (n=46) after completing baseline measurement in June 2022.

Exercise program

The 4-week MVPA intervention engaged in supervised aerobic exercise, resistance training and interesting sport for 60 minutes, 3 days/week (Monday, Wednesday and Friday). Participants completed four sessions including preparation module (15 min), training module (30 min), and relaxation module (15 min). Another aerobic module (15 min) was added in each Friday. Children in intervention group

were also encouraged to take exercise for 10 minutes together with their parents at home two hours later after dinner including ten minutes' walking (at least 1000 meters) or at least 200 times rope skipping. Parents were invited to come to school at the beginning of the intervention program to accept project training. In order to safeguard the rights of the control groups, the same physical activity intervention was given to the control group at the end of the research.

Strength was measured by Tri-axial body motion recorder (ActiGraph, Wgt3x-BT, USA) to maintain relative intensity. Children were required to wear ActiGraph by a professional investigator on the left wrist, loosely tightened, and required to wear it at all times except for the shower during the intervention for 7 days. Valid data for each day were defined as: recording a wearing time of more than 8 hours, excluding sleep time, and recording counts per minute (CPM) of no more than 20 minutes of continuous 0 CPM. After the test, the raw data were analyzed using Actilife V6.13.4 software, and after extracting and calculating the daily average MET levels, CPMs per minute greater than 20,000 were excluded from the data, and the average daily total MVPA time was accumulated and calculated based on different physical activity intensity cut-offs (total MVPA time = total VPA time + total MPA time). The vector data recorded by the ActiGraph were transformed into different physical activity intensity times with cut-off points of 100 CBM for light physical activity, 2296 CPM for moderate intensity, and 4012 CPM for high-intensity activity, based on Evenson et al.'s [15] study about the proposed cut-off points.

At baseline, Physical Activity Questionnaire for Children (PAQ-C) was answered

by each participant. The PAQ-C reflects the overall physical activity level of children and adolescents in the past 7 days and the MVPA levels[16]. The scale consists of 10 items, 9 of which are structured to investigate MVPA levels in children and adolescents. A 1-5 scale was used to obtain a total score by summing the scores of the individual items. The PAQ-C scale scores < 2.33 defined as low physical activity levels, 2.33-3.66 as moderate physical activity levels, and >3.66 as high physical activity levels[17].

Obesity outcomes

Height was measured to the nearest 0.1cm using Height Measuring Ruler (Selcom, Germany). Measurement was taken on the vertical distance from the apex of the skull to the ground in the standing position. Body weight and fat mass was measured to the nearest 0.1kg using Body Composition Analyzer (DC-13, Parade, Japan). Subjects were measured barefoot, wearing light. Waist circumference was measured to the nearest 0.1cm with circumference measuring tape. Measurement was taken between the lowest rib and the iliac crest with the abdomen relaxed at the end of a gentle expiration. Hip circumference was measured to the nearest 0.1cm with circumference measuring tape. Measurement was taken on the most prominent part of the buttocks and wraps it horizontally around the buttocks.

Preperitoneal fat and abdominal subcutaneous fat thickness were measured by ultrasonography. The thickness and area of preperitoneal fat and abdominal subcutaneous fat were measured with a linear array probe L12-5 (38 mm, 5-12 HZ) according to the method of Suzuki[18]. The linear array probe was placed vertically in

the median position of the upper abdomen, and the probe was moved 1-5cm above the umbilicus to obtain an image that included the maximum preperitoneal fat thickness. The probe was then placed vertically in the abdominal white line to obtain a transverse image. The preperitoneal fat thickness is the maximum height in the triangle. Abdominal subcutaneous fat thickness is the height above the triangle.

Blood pressure was measured by medical sphygmomanometer for 3 times. The subjects were at rest for more than 15 minutes. The blood pressure was measured on the right arm in a seated position with the elbow, sphygmomanometer and heart at the same level. All subjects were fasted for more than 12 hours. 3 ml of non-anticoagulated blood and 2 ml of EDTA anticoagulated blood were collected. The same medical examiner examined before and after the intervention to ensure data comparability.

Genotyping

The whole genomic DNA of blood mononuclear cells was performed using a fully automated nucleic acid extractor (QIAGEN, QIAcube, Germany), the DNA extraction reagents were used in a magnetic bead method DNA extraction kit (QIAGEN, 51331, Germany), and the detailed steps were performed according to the reagent and instrument instructions. 14 SNP loci associated with obesity and BMI were reliably validated based on GWAS studies in large Asian populations[19, 20]. Genome-wide typing of the 14 targeted SNP loci was performed using the MassARRAY® system (AgenaBioscience Inc., USA) based on MALDI-TOF mass spectrometry for typing each locus. Due to the differences in the contribution effect of

each allele to obesity or BMI, a weighted genetic risk score (WGRS) was used to assess the genetic susceptibility to obesity in this study, calculated as follows:

$$WGRS = (\beta_1 \times SNP_1 + \beta_2 \times SNP_2 + \ldots + \beta_{14} \times SNP_{14}) \times (\text{total SNP allele points / sum of } \beta\text{-effect coefficients})$$

where β_n coefficient is the contribution of each effect locus to BMI and obesity found in the GWAS study.

Covariates

Children's gender, age, date of birth, ethnicity and household income were collected in questionnaires answered by children at baseline. Non-quantitative Food Frequency Questionnaires (FFQ) was used to collect information on the dietary frequency, including 24 types of daily dietary foods such as cereals, flour, meat from livestock, seafood, vegetables, milk, snacks, and beverages. Dietary pattern factor score was calculated for each participant and included as covariate[21].

Statistical Analyses

Data from the questionnaires and physical examination data was entered using EPIDATA 3.0 software, with trained entry clerks double-entering independently. All analysis was processed using SPSS 20.0 software, and a two-sided *P* value of less than 0.05 indicated a statistical difference with a test level of α=0.05.

Baseline participant characteristic differences between intervention groups were evaluated by independent t-tests for continuous variables and chi-squared (χ^2) tests for categorical variables. The three possible genotypic frequencies for each gene studied were evaluated by Chi-Squared tests and found to be in Hardy–Weinberg equilibrium.

Associations between WGRS and obesity outcomes were analyzed using generalized linear models. Generalized linear models (GLM) were established to analyze two main effects of interest (gene, MVPA) and one interaction (gene ×MVPA) on each obesity outcome. Change in obesity outcome served as dependent variables. Covariates included age, sex, ethnicity, and family income status. Estimated marginal means of gene × MVPA interaction, adjusted by the covariates, are presented in figures.