The effect of “living high-training low” on weight loss in overweight Chinese adolescents: study protocol for a randomized controlled trial

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Abstract

Background

Lifestyle modification including a healthy diet and increased physical activity is the cornerstone of the treatment of obesity in obese children and adolescents. However, compensatory changes in appetite and energy expenditure elicited by exercise and dieting make it hard to maintain the reduced weight over the long term. Anorexic effect of hypoxia can be potentially utilized to counteract the compensatory increase in appetite in a traditional weight loss program, thereby enhancing the success of weight loss in the long term. The purpose of the study is to assess the effectiveness of four week intermittent hypoxia exposure added to a traditional exercise and diet intervention in inducing short- and long-term weight loss in obese adolescents.

Methods/Design

A randomized controlled clinical trial will be conducted in the study. We will recruit 40 adolescents aged 11 to 15 years from the summer weight loss camp at the Shanghai University of Sport. The participants will be randomly assigned to two groups, i.e., the control group and the hypoxia group. The control group will exercise, eat a balanced diet and sleep in normal condition, and the hypoxia group will exercise, eat a balanced diet and sleep in normobaric hypoxia chambers (sleep high and train low). Body composition will be measured using DXA at baseline, after 4-week intervention and at two months follow up. To investigate the mechanism for the weight loss effect of hypoxia, we will focus on the interactive effect of exercise and hypoxia on appetite regulatory gastrointestinal hormones including leptin, ghrelin, PYY, CCK and
GLP-1. The circulating levels of these hormones will be assessed at three time points (i.e., baseline, post-intervention, and follow-up) and their changes will be analyzed for the associations with changes in body composition.

**Discussion**

Our study will be the first to evaluate the effectiveness of “sleep high and train low” on short- and long-term weight loss among obese adolescents. A potential mechanism for the appetite regulatory effect of hypoxia will also be explored. The results of the study will provide evidence-based recommendation for the hypoxia use in weight loss intervention among obese children and adolescents. Furthermore, the clarification of mechanisms leading to weight loss in “sleep high and train low” might provide information for the development of new strategies in combating obesity in the future.

**Trial registration**

Name of trial register: Effects of hypoxia and exercise on the immune cell function in overweight adolescents

Web address of trial register: http://www.chictr.org/cn/

Date of registration: 2014-01-10

Number of registration: ChiCTR-TRC-14004106

**Keywords:** hypoxia; living high-training Low, weight loss; adolescents; gastrointestinal hormone
Background

Obesity is a global pandemic, and its prevalence among children and adolescents has also increased worldwide, becoming a serious public health problem [1]. In China, nearly 215 million people are overweight or obese, and 12% of them are children (age<17 years), as estimated by the 2002 China National Nutrition and Health Survey [2]. Obesity in childhood has been associated with an increased risk for diabetes, hypertension, cardiovascular diseases and various cancers in adulthood [3, 4]. Therefore, preventing and treating obesity in childhood and adolescence is crucial.

The causes of childhood obesity are complex and multifaceted involving genetic factors, environmental and behavioral factors. The current worldwide epidemic of obesity is believed to be attributable to the modern living environment, which promotes a sedentary lifestyle and excessive consumption of calorie dense food. Accordingly, a lifestyle modification including healthy diet and increased physical activity has been recommended as the cornerstone of prevention and treatment of obesity. Physical activity increases energy expenditure, and in combination with a healthy diet, it is effective in inducing weight loss. However, the reduced weight achieved through weight loss program is hard to maintain over the long term. The failure to achieve long-term weight loss is believed to be caused by compensatory changes in appetite and energy expenditure elicited by exercise and dieting. Currently, a complete understanding of the relationship between exercise, appetite regulation and weight management is lacking.
Recent research revealed that appetite-regulating hormones might play an important role in moderating the interrelationship between exercise/dieting, appetite and weight regain. Among various potential appetite-regulating hormones, the gastrointestinal hormones, ghrelin, peptide YY (PYY), cholecystokinin(CCK) and glucagon-like peptide-1 (GLP-1) are well studied. Ghrelin is the only hormone that has been shown to be orexigenic, while PYY, CCK and GLP-1 are satiety regulatory hormones [5, 6]. These hormones are episodic hormonal signals occurring in unison with episodes of eating. They signal satiation and satiety either via the vagus nerve (which connects the gut to the brain) or via blood perfusing the hypothalamus.

It is accepted that in response to weight loss, counter-regulatory adaptations develop in the appetite regulatory system including the gastrointestinal hormones, defending impositions that promote a negative energy balance. Different weight loss intervention approaches are likely to cause different counter-regulatory adaptations in terms of the content and the magnitude of the response. There is some evidence suggesting that diet-induced weight loss is associated with a compensatory increase in total ghrelin (GT) plasma levels and a blunted postprandial release of PYY and GLP-1 [7, 8]. Exercise-induced weight loss may increase the drive to eat, as shown by increased levels of acylated ghrelin (AG) and subjective feelings of hunger in fasting, but it may also improve satiety as evidenced by an increase in the late postprandial release of GLP-1 after exercise training [9].
Few studies have investigated the combined effect of exercise and dieting on appetite in children and adolescents. Based on the limited data, it appears that ghrelin levels increase after weight reduction program, while no change in PYY levels [10-13]. Similarly, we have observed an increase in ghrelin concentrations after weight loss in adolescents, who participated in an exercise and diet intervention for four weeks in a summer camp program in our previous study (unpublished data).

Recently, the effect of high altitude on appetite regulation has attracted researchers’ great attention. It is a widely observed phenomenon that a high altitude can induce loss of appetite [14-16]. In many studies, loss of appetite and the resulting decrease in energy intake have been attributed to acute mountain sickness (AMS), symptoms of which include headache and anorexia. However, loss of appetite cannot be merely a by-product of AMS because anorexia and weight loss still persist when symptoms of AMS have subsided [17]. Furthermore, studies conducted in normobaric hypoxia chambers, where other environmental stressors associated with high altitude can be eliminated (i.e., extreme cold and physical exertion), demonstrated that hypoxia perse can cause reduced appetite and energy intake, and loss of body weight [18]. It has been suggested that the effect of hypoxia on appetite is mediated by the changes in gastrointestinal hormones [19-22].

It is perceivable that to promote success in long term weight loss, hypoxia can be implemented in a traditional diet and exercise weight loss program, because the
negative effect of hypoxia on appetite might be able to balance the positive effect of diet/exercise. Exercise and hypoxia have been used in combination, in sports to induce maximal increase in aerobic endurance in athletes, but rarely been used in weight loss, especially in children and adolescents. We have run several sessions of weight loss summer camps designed for obese adolescents in Shanghai China. The effect of exercise and hypoxia on weight loss has been explored in our preliminary studies and interesting results have been generated [23, 24]. Now we designed a randomly controlled trial investigating systematically the long-term weight loss effect of exercise and hypoxia in obese adolescents and the mediating effect of the gastrointestinal hormones. We hypothesize that 1) exercise and hypoxia have an additive effect in weight loss via increasing energy expenditure and suppressing appetite; 2) hypoxia use would lead to less rebound of weight loss after intervention, due to its potential negating effect on compensatory changes in appetite elicited by exercise and diet in a traditional weight loss program; 3) changes in gastrointestinal hormones (including ghrelin, PYY, CCK and GLP-1), as well as in cytokine IL-6, would be associated with changes in body weight after intervention and during follow-up.

**Aims**

The aims of this randomized controlled trial (RCT) are to 1) evaluate the effectiveness of intermittent hypoxia and exercise, in combination with a balanced diet, in inducing short- and long-term weight loss in Chinese children and adolescents, and to 2)
determine the molecular mechanisms behind the benefits of hypoxia in enhancing weight loss.

Methods/Design

Design

This study is a randomized controlled clinical trial with two parallel arms involving 40 obese adolescents. To assess the effectiveness of 4-week intermittent hypoxia added to a traditional exercise and diet intervention in inducing short (after 4-week intervention) and long term (at two months follow-up) weight loss in obese adolescents, we will recruit 20 adolescent boys and 20 girls (aged 11 to 15 years) from our summer weight loss camp. They will be stratified according to gender and randomly assigned to two groups, i.e., the control group, who will exercise, eat a balanced diet and sleep in normobaric condition, and the hypoxia group, who will exercise, eat a balanced diet and sleep in normobaric hypoxia chambers (sleep high and train low). Outcome assessment and data analysis will be performed by professionals who are blinded to the group assignment of subjects.

Sample size estimation

Sample size estimation in this RCT is based on the expected fat loss in 4-week of hypoxia exposure plus exercise and dieting. The data of our preliminary experiment showed that the means of fat percentage decrease in normoxia and hypoxia group was 3.1% and 6.0% respectively, and the standard deviation of the change was about 3%. It is estimated that a sample size of 17 participants per group will be required to
observe a similar result for an 80% power. Considering a 15% dropout and exit rate, we will recruit 40 subjects with 20 in each group.

**Ethical approval and consent**

The study will be conducted according to the principles expressed in the Declaration of Helsinki. The study protocol has been approved by the institutional review board at the Shanghai University of Sport (reference number: 2014 Ethics Approval Note 1). Participants in this study are volunteers. None of the measurements is known to entail any significant health risk, nor is the intervention. The study has its own physician to ensure the eligibility and safety of all participants. All data will be handled and archived confidentially. The benefits and associated risks of the study will be carefully explained and the voluntary nature of participation will be emphasized. Informed consent and assent will be obtained from all participants and their parents. Participants and their parents will have the option to end the participation at any stage if they so wish. If the physician and the principal investigator believe that there are risks of serious adverse events in the study, the trial will be stopped. The trial is registered with Chinese Clinical Trial Registry as ChiCTR-TRC-14004106.

**Subject Recruitment**

Obese adolescents aged 11 through 15 years will be recruited from the children registered for the 2014 summer weight loss camp at the Shanghai University of Sport. A public health nurse will assess the Tanner stage of each subject using the Tanner grading system [25, 26]. Obesity will be defined based on body-mass index (BMI),
calculated as weight in kilograms divided by height in meters squared (kg/m^2).

Although BMI ≥ 25 kg/m^2 and ≥ 30 kg/m^2 are international cut-off points for overweight and obesity, for many Asian populations, individuals with a BMI ≥ 23 kg/m^2 are considered to be at increased risk and those with a BMI ≥ 27.5 kg/m^2 at high risk [30]. Thus, we chose BMI ≥25 kg/m^2 as a criterion to recruit obese adolescents.

Adolescents will be excluded if they have concomitant renal, hepatic, or cardiac disease, and/or are being treated with drugs that could affect body weight and appetite (such as Orlistat, Lorcaserin, and phenterminetopiramate, as well as appetite suppressants).

**Randomization procedure**

The random allocation of adolescents to hypoxia or normoxia group will be conducted through a computerized randomization program by an independent statistician. Randomization will be performed only after a participant is confirmed to be eligible and written informed consent has been obtained. The randomization form will be completed and returned to the principal investigator. In order to minimize the potential bias, the exercise physiologist and the dietitian who manage the exercise training and the diet intervention will be blinded as to whether a subject will sleep in the hypoxia chamber or not. Hypoxia chamber will be prepared by an independent researcher. The researcher performing outcome assessments will be blinded to the subjects' intervention allocation.
**Intervention**

All the subjects, including both normoxia (control) and hypoxia group, will undergo four weeks of aerobic exercise training and dieting (eat a balanced diet). In addition, normoxia group will sleep in a normal condition while hypoxia group will sleep in a normobaric hypoxic chamber every night. All the measurements will be conducted two days prior to and post the intervention, and repeated two months later after the intervention (two months follow-up). Fasting blood samples will be collected in the morning of the testing day. In order to maximize the compliance and avoid the occurrence of any accident, the intervention will be closely supervised by a physician, a dietitian, and an exercise physiologist.

**Exercise training**

The same aerobic exercise training will be applied to both the normoxia and the hypoxia group. To promote participants’ interest, the exercise training will consist of three different activities including swimming (intensity: 6 MET), aerobic exercise (intensity: 7.5 MET), and basketball (intensity: 6 MET). Participants will exercise six days per week, twice daily, 1 h per session. The intensity of the exercise will be estimated using MET score. MET, the unit of energy expenditure, will be obtained by dividing oxygen uptake values (ml·kg\(^{-1}\)·min\(^{-1}\)) by 3.5 (1 MET is defined as resting metabolic rate that is 3.5 ml·kg\(^{-1}\)·min\(^{-1}\)). \(\text{VO}_2\) will be measured using pulmonary function equipment, Cosmed (K4b2, Italy) according to the manufacturer’s instructions.
**Diet modification**

All participants will receive well-defined and balanced daily meals during the 4-week intervention. Dietary recommendation will be individualized based on his/her basal metabolic rate and range from 1 600 to 2 000 kcal/day. The basal metabolic rate will be calculated using the Mifflin equation[27]. The caloric intake will be calculated based on the Chinese food chart. Each day, **three** well-balanced meals will be provided with the following calorie allocations: breakfast 35 %; lunch 40 %; and dinner 25 %. Each meal comprises 30% protein, 20% fat and 50% carbohydrate by energy. Animal and vegetable oil and starch-rich food will be minimized, while the intake of vegetables, fruits, bean products, rabbit meat, beef, pork, and cellulose will be increased. This prescribed diet includes pivotal nutrients such as vitamins, minerals, essential amino acids, fibre, and polyunsaturated fatty acids.

**Hypoxia exposure**

To test the hypothesis that hypoxia exposure would ameliorate the compensatory increase in appetite elicited by exercise and diet, experimental group (hypoxia group) will sleep in a normobaric hypoxia environmental chamber every night during the 4-month intervention. The advantage of using environmental chamber, as compared to real altitude situations, is that the effects of hypoxia can be isolated from the influence of other confounding factors present in real altitude situation, such as the influence of temperature, humidity, and physical activity levels. The normobaric hypoxia will be designed to mimic an altitude of 2700m. We will use large hypoxic
training system (German Low Oxygen company) of hypoxia test laboratory of Shanghai Oriental Oasis Training Base to simulate hypoxic environment (14.7% O2; ~2700m). After a 1-day hypoxia acclimation period, participants of the hypoxia group will sleep in hypoxia training laboratory for 10 hours every night (from 21:00~7:00 next day), 7 times/wk, for 4 wks.

**Attrition and Compliance**

Due to the voluntary nature of the enrollment of the summer weight loss camp, attrition rates will be very low. In a pilot trial involving 50 overweight, male Chinese adolescents, 47 children completed a 4-week diet and exercise intervention, and only 3 children (6%) dropped out of the study due to loss of interest. Our recruitment plan was made based on this attrition rate.

**Assessments**

Baseline assessments will be conducted two days before the onset of the 4-week intervention. Post-intervention and two months follow-up assessments will be conducted 2 days and 2 months after completion of the intervention respectively. The primary outcome of the study will be body composition, and the secondary outcome measures will include appetite score, and blood levels of gastrointestinal hormones including leptin, ghrelin, PYY, CCK and GLP-1, as well as circulating levels of IL-6. All the assessments will be performed in the laboratory of exercise physiology at the Shanghai University of Sport.
**Body composition**

Body mass and height will be measured using a digital scale (Yaohua Weighing System Co., Shanghai, China) and a wall-mounted stadiometer (TANITA, Tokyo, Japan), respectively. Body composition and fat distribution will be measured using dual energy X-ray absorptiometry (DXA) (GE Lunar Prodigy). The software (ENCORE, version 10.50.086) will be used to analyze total lean mass (TLM), total fat mass (TFM), total body fat percentage (%TBF), android fat percentage (%AF) and gynoid fat percentage (%GF).

**Cardiorespiratory Fitness**

Peak oxygen consumption (VO$_{2\text{peak}}$) for all participants will be measured using the Cosmed (K4b2) portable metabolic system. Expired respiratory gases will be collected on a breath-by-breath basis during a submaximal treadmill (H/P/Cosmos Pulsar 4.0, Germany) test. Exercise will start at a speed of 2 km/h, which will be increased every 2.5 minutes by 1 km/h till 8 km/h is reached. 80 % of the maximum heart rate (HR$_{\text{max}}$) will be set as a criterion of exercise termination. Trained research assistants will record heart rate and power output data at the end of each stage. Heart rate will be measured using polar heart rate monitor.

**Blood analyses**

Fasting blood samples (2 mL) (12-h fasting) will be obtained at baseline,
post-intervention, and at 2 months follow-up. Serum levels of gastrointestinal hormones including leptin, ghrelin, PYY, CCK and GLP-1, as well as cytokine IL-6, will be measured using commercially available ELISA kits (R&D Systems). As reported, the lower limit of sensitivity of the assays will range between 0.8pg/mL~10pg/ml. The intra-assay coefficient of variation (CV) will be less than 5% and the inter-assay CV will be less than 10%. Absorbance will be read at 450 nm wavelength using a microplate reader (Bio-rad 550, Bio-Rad, Hercules, CA).

**Appetite assessment**

Children’s appetite at baseline, during and post-intervention, and during follow-up will be assessed via a simple 8-item appetite questionnaire (appendix A). The questionnaire was developed by modifying the 8-item Council on Nutrition appetite questionnaire (CNAQ). CNAQ is a short, simple appetite assessment tool, which has been validated and proved to be able to predict weight loss in community-dwelling adults and nursing home residents [28]. We modified the CNAQ reflecting the nature of our intervention.

**Data management and statistical analysis**

Data generated in the study will be collected and summarized with respect to subjects’ baseline characteristics. Group differences in demographical and clinical characteristics at baseline will be tested using a two-sample t-test for quantitative data and chi-square test for qualitative data. Repeated Measures Analysis of Variance (RM
ANOVA) will be used to analyze the main effect of treatment and potential time-by-treatment interactions. Multiple multivariate regression analysis will be performed to analyze the relationship between changes in body composition, appetite score and gastrointestinal hormones following intervention and during follow-up. All statistical analyses will be performed using the Statistical Package for the Social Sciences (SPSS, Inc., Chicago, IL, USA) for Windows version 18.0, and a significance level of 0.05 will be used.

Discussion

To the best of our knowledge, our study will be the first to evaluate the effectiveness of “sleep high and train low” on short- and long-term weight loss among obese adolescents. The study will focus on obese adolescents due to the rising prevalence of obese children in China and the adverse impact of childhood obesity on adult health. Adolescent obesity has been attributed mainly to a sedentary lifestyle and unhealthy diet. In China, great effort has been made to use exercise and dietary intervention to treat obesity and metabolic disorders in children. However, the weight rebound following most of the weight loss programs has made such an effort fruitless. The weight rebound is believed to be caused by the compensatory increase in appetite induced by exercise and diet. Perceivably, the agent that can counteract this compensatory effect will benefit obese patients in achieving long-term weight loss. Hypoxia is potentially one of such agents: anorexia effect of hypoxia has been widely observed and might be utilized to dampen the appetite compensatory effect of
exercise and diet, thereby promoting the success of weight loss in the long term. Due to the profound benefits of exercise training and diet in the obese population, we don’t expect to use hypoxia as a replacement, but a supplement of exercise and diet intervention. Therefore, we will use children undergoing exercise training and diet but at normoxia as control, in order to evaluate the value of hypoxia in promoting weight loss, and minimizing weight rebound after intervention.

Instead of using continuous hypoxic exposure such as at high altitude, we choose to use intermittent hypoxic exposure, which has been recommended as an option for free-living obese population [29]. The effect of intermittent hypoxia exposure on weight reduction has been reported previously. In a model of diet-induced obese mice, it has been shown that intermittent hypoxic exposure induced decreases in body mass, blood glucose levels, and cholesterol erythropoietin concentrations [30, 31]. In a few human studies, the synergistic effect of exercise and hypoxia in treating obesity and associated metabolic disorders have been demonstrated, where low intensity exercise training at normobaric hypoxic condition (15% O₂) led to more weight loss and greater improvement in metabolic health, as compared to training at ambient condition (21% O₂)[32-34]. However, further evidence-based studies are needed to carefully evaluate the therapeutic value of hypoxia and the model of utilization in treating obesity, especially in children. Intermittent hypoxia exposure, as used in the “sleep high and train low” paradigm has been proposed for endurance athletes to maximally enhance their endurance exercise capabilities, however the clinical use of
intermittent hypoxia in the management of cardiometabolic diseases has rarely been tested. Our study will be the first to evaluate the effect of the “sleep high and train low” on weight loss in obese adolescents. Through monitoring a wide spectrum of health indices including blood pressure, heart rate, vital capacity, blood glucose and insulin levels, blood lipid profile, as well as immune function, we will careful monitor any possible side effect associated with hypoxia exposure.

Meanwhile we will investigate the mechanism for the effect of hypoxia on weight management. We will focus on the regulatory effect of “sleep high and train low” on gastrointestinal hormones levels. In addition, we hypothesize that the cytokine, IL-6 might also play a role in mediating the appetite regulatory effect of exercise (positive effect) and hypoxia (negative effect). The clarification of mechanisms leading to weight loss in “sleep high and train low” might provide information for the development of new strategies in combating obesity in the future.

**Trial status**

Pilot study involving a small group of overweight children has been finished based on the summer weight loss camp in our institution in 2013. The intervention modality has proven to be tolerated very well among children and effective in inducing significant weight loss. For the official trial, participant recruitment will start in April 2014. Baseline measurements will be taken in June 2014, and the 4-week intervention will be completed by August 2014, which will be followed by a two month follow-up.
Feedback on the preliminary results of participants’ health status will be provided to the participants upon completion of the study.
List of abbreviations:

LHTL, living high-training Low; TG, total ghrelin; AG, acylated ghrelin; PYY, peptide YY; CCK, cholecystokinin; GLP-1, glucagon-like peptide-1; AMS, acute mountain sickness.
Competing interests:
The authors declare that they have no competing interests.
Authors' contributions:

RW and PC conceived of the study, and participated in its design and coordination and drafted the manuscript. WX, NW and BG AB carried out the pilot study and helped in the design of the current study. DL and XW participated in the design of the study and helped to draft the manuscript. All authors read and approved the final manuscript.
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Figure legend:

Figure 1. Flow of participants through the trial.

Additional files:

Additional file 1. Appetite questionnaire
Obese adolescents screened (n = 40)

Baseline assessment
2 days before the onset of the intervention

control group (n = 20)
exercise, eat a balanced diet and sleep in normobaric condition

hypoxia group (n = 20)
exercise, eat a balanced diet and sleep in normobaric hypoxia chambers

Post-intervention assessment
2 days after the 4-week intervention

Follow-up assessment
2 month after the intervention

Figure 1. The flow of participants through the trial.
Additional files provided with this submission:

Additional file 1: Additional File 1.docx, 15K
http://www.trialsjournal.com/imedia/4539000611907888/supp1.docx