Effects of lutein supplementation on inflammatory biomarkers and metabolic risk factors in adults with central obesity: study protocol for a randomized controlled study

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Lutein supplementation, central obesity, inflammatory biomarkers, metabolic risk factors.
Abstract

Introduction: The prevalence of central obesity is sustained growth and visceral fat is associated with increased production of inflammatory factors and metabolic risk factors. Lutein might retard the development of the metabolic disease through the antioxidant and anti-inflammatory properties. Furthermore, epidemiological studies associate higher dietary intakes and serum levels of lutein with decreased adiposity. However, few of the randomized controlled trails (RCT) have showed the effects of lutein supplementation on inflammatory biomarkers and metabolic risk factors, especially in adults with central obesity. Methods and analysis: This study will be conducted as a double-blinded, and parallel placebo-controlled clinical trial in which 120 central obesity people aged 18-60 years old and willing to provide informed consent will be randomly assigned to intervention and placebo group in a 1:1 ratio according to gender, age and waist circumstance. The intervention group will receive daily lutein 10 mg supplementation for 12 weeks to explore the effect of lutein supplementation on serum lutein, glycemic and lipid profiles, inflammatory factors and body composition. Two population (intention-to-treat population and per-protocol population) will be used in the data analyses. Discussion: Our findings from this trial will contribute to the knowledge of the association between lutein supplementation and inflammatory biomarkers, metabolic risk factors in people with central obesity, which will offer us a possibility for the prevention of inflammatory diseases. Trail registration: Chinese Clinical Trial Registry, ChiCTR1800018098. Registered on 30 August 2018. Keywords: Lutein supplementation, central obesity, inflammatory biomarkers, metabolic risk factors.

Introduction

The prevalence of obesity is sustained growth and has reached epidemic proportions. WHO
estimated that, by 2016, 39% of adults aged 18 years and over were overweight, and 13% were obese in the world [1]. Obesity represents a major risk factors for an expanding set of chronic diseases such as type 2 diabetes mellitus, hypertension, cardiovascular diseases and cancer [2-4]. It is also accompanied by increased risk of mortality, for body weight mass (BMI) over 25 kg/m², the mortality increased approximately log-linearity with BMI [5]. Substantial literatures suggested that oxidative stress and inflammation may be mechanistic link between obesity and metabolic diseases [6].

It is clear that the levels of oxidative stress and the inflammatory factors increased as the adipose tissue increased. While, the adipokines (interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), adiponectin, leptin and resistin) secreted by adipose tissue play role in the homeostatis of various physiological process, such as the insulin sensitivity, energy expenditure and fatty acid oxidative [7, 8]. Compared to deposits in other fat tissues, however, visceral fat creates more adverse effects including higher levels of free fatty acid flow, inflammatory molecules, and adipocytokines [9-12]. Epidemiological studies pointed that visceral fat was correlated with insulin resistance and glucose intolerance as well as increasing levels of C-reactive protein (CRP), TNF-α, IL-6, isoprostanes and monocyte chemoattractant protein-1(MCP-1)[12, 13].

Lutein, an oxygenated carotenoids found primarily in dark green leafy vegetables and egg yolks. Lutein is mostly known for its effects on visual function and its preventative effect against macular degeneration, potentially acts as an antioxidants that protect against light-induced oxidative damage in the retina by oxygen radicals [14, 15]. Given it beneficial antioxidant and anti-inflammatory properties [15], it is hypothesized that lutein may have beneficial effects on inflammatory biomarkers and metabolic risk factors. Xu et al.[16] gave the early atherosclerosis patients with lutein supplementation and found that the intervention group had a significant decrease in serum IL-6 and MCP-1. Another
intervention study conducted in healthy nonsmokers found the lutein supplementation increase total antioxidant capacity and reduce CRP [17]. Although accumulating epidemiological studies have shown an inverse relationship between serum carotenoids concentration and a circulating markers of inflammation such as the CRP and soluble intercellular cell adhesion molecule-1 (ICAM-1), intervention studies has focused mostly on other carotenoids, such as lycopene [18, 19], less on lutein.

Another hypothesis is that lutein may have an anti-adiposity action. Our previous study found that lutein treatment significantly decreased body weight as well as abdominal and total adipose tissue, which were increased by high fat diet feeding in mice [20]. Besides, the concentrations of total cholesterol (TC) and triglyceride (TG) in serum and the liver as well as the serum levels of low-density lipoprotein cholesterol (LDL-C) were significantly reduced [20]. In addition, epidemiological studies associate higher dietary intakes and serum levels of lutein with decreased adiposity [21-23]. Despite the animal studies and human epidemiological studies linking lutein to adiposity, however, there is a paucity of randomized controlled trials (RCT) utilizing lutein supplementation to assess changes in adiposity.

An important aspect to consider with lutein supplementation is that it cannot be synthesized by human and only can be obtained through diet or supplements. However, our previous study found that the medians intake of lutein (2.499mg/d) is far below than the special proposed levels (SPL: 10mg/d), which is advised in Chinese Dietary Reference intakes 2013[24]. In addition, although antioxidant properties of lutein have been proven in different population, reports dealing specifically with lutein in people with central obesity are scarce. In light of the information above, giving central obesity people with lutein supplementation is worthy consideration.

Methods
**Study design and setting**

This is a double-blind, parallel randomized controlled trial for central obesity participants. The Standard Protocol Items: Recommendations for Interventional Trials (SPIRIT) 2013 statement will be followed in this trial (Additional file 1) [25]. The proposed clinical trial will be conducted at the Community Health Service Centre (CHNS) in Nanshan District, Shenzhen, Guangzhou Province, China, for 12 weeks to assess the effects of lutein supplementation (10 mg/d) on inflammatory biomarkers and metabolic risk factors in adults with central obesity. Figure 1 illustrates the overview of the study.

**Study participants and eligibility criteria**

It was estimated that 52 participants per treatment group would be required for 80% power and 5% level of significance to detect the change of serum TG over 12 weeks of supplementation [26]. Allowing for a dropout rate of approximately 15%, we aim to recruit 120 participants in total. Participants were eligible for this study if they fulfil the following criteria (1) aged between 18 and 60 years; (2) waist circumstance: male ≥ 90 cm and female ≥ 85 cm; (3) deficient in lutein intake (dietary lutein intake < 10 mg/d) or low levels of serum lutein; (4) willing to sign the informed consent. People with any of the following will be excluded from participation: (1) using lutein supplementation or related health care products with 1 month; (2) using antibiotic in 1 month; (3) secondary obese patients; (4) those who had a special diet or meal replacement for early 2 weeks; (5) using insulin or insulin sensitizers in 1 month; (6) people with chronic diseases (including type 2 diabetes, hypertension and dyslipidemia) over 10 years; (7) patients with hepatorenal dysfunction (creatinine > 1.2 times the upper normal limit of creatinine, aspartate aminotransferase > 1 times the upper normal limit); (8) any active tumors/cancers; (9) Regular use of drugs affecting blood lipid; (10) Pregnant or lactating women; (11) history of gastrointestinal surgical operation;
Randomization and blinding

When all the inclusion and exclusion criteria have been executed and informed consent have been obtained, participants will be allocated to lutein group or the placebo group. Computer-generated random numbers will be used for allocation sequence generation with stratification completed according to gender, age and waist circumstance. This parts of work will be perform by a persons who will be operationally independent from the study team. Allocation concealment will be implemented by using the random number card in a black envelope. The investigator and patients will be blinded to the sequence and the treatment condition. Patients’ data collected will be kept confidential during this trial. The grouping of the study will be opened after all individuals complete the study protocol.

Supplements

The participants will be receive either 10 mg of lutein or placebo capsules per day for 12 weeks. Lutein were suspected in soybean oil and given in acid soluble gelatin capsules and the placebo containing soybean oil (100%). To maintain and guarantee blinding, the capsules will be identical in appearance, shape, smell and weight. All capsules will be provided by the Royal DSM N.V. (Beijing, China). Face to face instruction on how to take the capsules will be provided at the baseline. Enrolled participants will be required to: (1) take one capsule along with meals daily; (2) bring the remaining capsules in the bottle to the next visit; (3) avoid to make any changes in dietary habits, such as suddenly eating plenty of foods rich in lutein. (4) avoid taking any health care products during follow-up.

Study procedures

Recruitment and screening

Table 1 provides the schedule for enrollment, intervention and follow-up. Participants will be recruited by placing advertisements on social media sites and community notice boards. Once the volunteer expresses initial interest in participating in the study, he/she
will have a face to face or telephone communication with the research assistant. Detailed information on the study trial including the purpose, the way of interventions and durations will be told to the volunteers. If they are still interest in this project after careful consideration, the structured standardized screening questionnaire, which is mainly around the inclusion and exclusion criteria for the study, will be used to assess the potential eligibility of the participants for the study. Potential participants who are found not to be eligible will be excluded at this stage. Eligible participants will be invited to attend the study for a further screening visit, which takes place at CHSC. Firstly, the informed consent will be obtained from all participants. Second, for safety assessments, the liver and renal function will be evaluated by collecting fasting blood samples and spot urine samples. Third, the anthropometric index will be measured by the trained investigators.

**Test visit**

In the test visit 1 (week 0), eligible participants are allocated to either a lutein supplement group or a control group. The lutein supplement group involves one 10 mg lutein capsule daily and the control group takes the placebo. They will be given the 6 weeks supply of capsules in the test visit 1 and visit 2, respectively, and required to return the remaining capsules in the next visit, which will be counted and recorded for each participant in order to ensure compliance with the protocol. Participants are defined as non-compliant if they have been taken less than 80% of the study product. Medication, health status or any recent changes around the inclusion and exclusion criteria since screening questionnaire are also re-assessed in order to evaluate whether participants are still suitable to participants in the study. Anthropometric measurements and blood sample will be collected at each visit, and stool sample, spot urine sample and dietary assessments will be collected at test visit 1 and test visit 3. At the test visit 1 and test
visit 3, the volunteers are asked to complete the short form international Physical Activity Questionnaires (IPAQ-short form) and Mini-mental State (MMSE). Throughout the intervention, each patient will be advised not to make any changes in her/his dietary habits and physical activity level (PAL). At the end of the each visit, participants are thanked for their time and will be provided the breakfast. After the test visit 2, people in the placebo will be given the same amount of lutein supplements as the intervention group. Full details of data collected at each time point can be found in Table 1.

**Anthropometric measurements**

Anthropometric measurements will be collected using standardized equipment and examination procedures. Participants will be weighted and heightened with light clothes and without shoes in the morning of the follow-up. Body fat percentage and total body water will be calculated using one-stop self-service monitoring machine (E-Techco Information Technologies Co., Shenzhen, China). Waist circumference (WC) is measured at the midpoint between the lower edge of the rib arch and the iliac crest while the subjects are standing with steady breathing. The hip circumference (HC) will be measured at the widest part of the buttocks at the intertrochanteric levels to the nearest 0.1cm. The other anthropometric indices will be calculated using the following equations: BMI=weight (Kg)/height (m$^2$); waist-to-hip ratio (WHR) = waist (m)/hip (m); waist-to-height ratio (WHtR) = waist (m)/height (m). Blood pressure (BP) will be measured using a standard mercury sphygmomanometer with a cuff placed on the upper right arm after a 5-min rest with a 1-min interval before subsequent measurements. Three BP readings are recorded, and the mean will be calculated. All anthropometric measures will be taken by trained research assistant using standard equipment according to the standard guidelines.

**Biological sampling**

At the baseline, middle and the end of the trial, 12 ml of venous blood sample (4 ml in
EDTA-coated sterile tubes and 8 ml in regular tubes) will be collected after fasting overnight. In addition to the indexes of liver and renal function (alanine aminotransferase (ALT) and aspartate aminotransferase (AST), creatinine), blood glucose, insulin and lipid profile (TC, TG, high-density lipoprotein cholesterol (HDL-C) and LDL-C) and CRP will be test on the day of the blood collection, the remaining samples will be divided and stored at -80°C freezers for long-term storage. The enzymatic methods (Biosino Biotechnology Company Ltd., Beijing, China) will be used for measuring serum hepatic enzymes, including the alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Serum creatinine will be determined using the Jaffe methods. Insulin concentrations (μU/mL) will be measured with Chemiluminescence microparticle immunoassay, high-sensitive CRP will be measured using the immunoturbidimetry assay, fasting blood glucose (FBG) (mmol/L) will measured by using the enzymatic colorimetric method with glucose oxidase using commercially. Insulin resistance (IR) will be estimated by homeostasis model assessment of insulin resistance (HOMA-IR): fasting insulin (μU/mL) *(fasting glucose (mmol/L)/22.5)[23]. Lipid profile will be determined by colorimetric methods using commercial kits (Biosino Biotechnology Company Ltd., Beijing, China) in Hitachi 7080 automated analyzer (Hitachi, Ltd., Tokyo, Japan). Serum lutein (μmol/L) will be analysed by high-performance liquid chromatography-tandem mass spectrometry method, retinol-binding protein 4 (RBP4) (μg/mL) and adiponectin (μg/mL) will be measured using the enzyme-linked immunosorbent assay (ELISA) kit (Adipogen, San Diego, CA, USA). Serum levels of MCP-1, TNF-α, IL-6, Interleukin-1β (IL-1β) will be measured using the flow cytometry method (FCM).

Participants will be required to provide the spot urine samples on the day of the screening, test visit1 and test visit 3. The routine urine test will be executed on the day of collection and the remaining samples will be randomly packed in 5-ml EP tube and then
stored for future analysis. Fecal samples will be collected at the test visit 1 and visit 3 using a stool specimen collection kit, which includes the operating instruction, an ice pack, gloves, one sterile container and spoon, sealed plastic pouch and a cool box. Participants can collect their fecal samples and bring them to the CHSC and then the samples will be transport and stored for further analysis.

**Dietary assessments**

At the screening periods, eligible participants will complete a questionnaire on food intake, which capture information on how much vegetables rich in lutein they intake, including the sweet potato leaves, spinach, broccoli, collards, leeks, lettuce and so on. This questionnaire provide a reference to roughly evaluate dietary lutein intake. At the baseline (week 0), middle (week 6) and the end of the trial (week 12), individuals will be required to provide the three days of 24-h recall through the phone or the internet. The data will collected by and calculated by the professional nutritionist according to the Chinese Food Composition Database (2009) and the United States Department of Agriculture Nutrients Database [27-28]. At each follow-up, questions on whether they are have changed their diets will be asked, including significant reduction or increase in dietary intake.

**Study outcomes**

The primary outcome is to measure changes to the inflammatory biomarkers between the intervention and placebo group from baseline (test visit 1) to 12 weeks later (test visit 2). The secondary objectives are to assess the change of the anthropometric index, including the weight, waist circumference, hip circumference and the body composition.

**Side effects and safety assessment**

There are no side effects of 10 mg/d of lutein supplementation. The observed safe level (OSL) of 20 mg/d for lutein has been suggested [29]. All participants can contact research
assistant at any time if they feel any uncomfortable. In addition, we will always pay attention to gastrointestinal symptoms and other adverse effects by phone calls or the internet. In case they develop severe side effects, they are advised to stop to take the supplementation. All serious and non-serious adverse events and/or reactions involving capsules supplementation are record by the research assistant. Individuals are reminded of the changes they have been asked not to make and they have been requested to record timely once these behavior had been changed.

**Statistical analysis**

Two population will be used in the analyses. The intention-to-treat (ITT) population includes all participants who have been randomized, while the per-protocol (PP) population contain all participants who accomplish the entire intervention. Baseline characteristics of the study will be summarized as means± standard deviations (SDs) for parametrically distributed data, geometric mean values (and 95 percent confidence intervals) for nonparametrically distributed data, and numbers (percentages) for categorical data, respectively.

Difference between participants who complete and withdraw from the trial will be analyzed using an independent t test or the Mann-Whitney test for continuous variables (e.g. age) and chi-square for categorical variables (e.g. gender). For primary and secondary outcomes, analysis of covariance will be used to examine differences between lutein supplement and placebo at 12 weeks, adjusting for potential confounding factors and effort modifiers (e.g. baseline age and gender, etc). Our statistical analysis will be performed by using R software and the results will be considered significant at $P \leq 0.05$.

**Data management**

Individuals’ data or other related information will be kept by in a located file at the principal investigator office. The authorized study team will enter the information into
electronic database using the double-entry methods. The private personal information (e.g. ID number and telephone) will be hidden and restricted to principal investigator. The electronic database will be unified managed by Shenzhen Nanshan Center for Chronic Diseases Control.

Discussion

This study was designed to test the hypothesis that lutein intervention will significantly modulate inflammatory factors and metabolic risk factors in people with central obesity, compared with a control group receiving the placebo capsules.

In designing this clinical study, several key decisions were made to overcome the current limitations in the published literature. Previous studies on the antioxidant and anti-inflammatory properties of lutein mainly focused on early atherosclerosis population [16, 30, 31]. However, as far as we are aware, well-controlled lutein supplementation is limited and this study will be the only study of its type to be conducted in central obesity. Central obesity is a state of chronic low-grade inflammation characterized by elevated concentrations of circulating inflammatory markers, which may be a critical link between metabolic disorders such as the insulin resistance and diabetes [11, 32], therefore, individuals with central obesity also deserve attention.

Seconds, when focusing on the central obesity, subjects with deficient in lutein intake were consideration. A larger body of research pointed that the dietary lutein intake and/or the serum lutein was negatively associated with adiposity [21-23]. In addition, a previous longitudinal study found that obese subjects had lower concentration of the total carotenoids (including the lutein) when compared with the subjects with a BMI less than 22kg/m$^2$ [33]. Which indicated that the central obesity may be need more antioxidant to protecting against oxidative stress and inflammatory generated by adipose tissue.
However, we need to confront that this clinical study has some limitations. First, the calculation of the dietary lutein intake was crude in the screening period. It would be a reference for us to choose these people who had a relatively lower intake. Second, this study include short-term impact of the intervention, for the participants will be treated for only 12 weeks. However, the development of metabolic diseases is a long and gradual process, and can be influenced by other factors. The improvements of the inflammatory biomarkers and metabolic risk factors do not suggest that lutein intervention can prevents metabolic diseases, however, it can offer us a possibility for the prevention of inflammatory diseases. Whether lutein intervention can prevent metabolic diseases needs the long-term intervention experiments.

**Trial status**

The trial commenced recruitment in June 2019 and is currently open for recruitment. Recruitment will cease when 120 participants have been randomized. It is anticipated that this target will be reached by August 2019.

**Abbreviations**

ALT: alanine aminotransferase; AST: aspartate aminotransferase; BP: blood pressure; BMI: body weight mass; CRP: C-reactive protein; FBG: fasting blood glucose; HC: hip circumference; HDL-C: high-density lipoprotein cholesterol; ICAM-1: Intercellular cell adhesion molecule-1; IL-6: interleukin-6; IL-1β: Interleukin-1β; IR: Insulin resistance; LDL-C: low-density lipoprotein cholesterol; MCP-1: monocyte chemoattractant protein-1; MMSE: Mini-mental State; RBP4: retinol-binding protein 4; TC: total cholesterol; TG: triglyceride; TNF-α: tumor necrosis factor-α; WC: waist circumference; RCT: Randomized controlled trial; WHtR: waist-to-height ratio; WHR: waist-to-hip ratio;

**Declarations**
Ethics approval and consent to participate

Ethics approval for this study was approved by the Shenzhen Nanshan Center for Chronic Diseases Control Ethical review committee (project: II20170014). The trial was registered on the Chinese Clinical Trial Registry (ChiCTR) on 30 August 2018 (ChiCTR Number: 1800018098). The study will be conducted in accordance with the ethical standards of the responsible Committee on Human Experimentation (institutional and regional) and the guidelines for the design, conduct, and reporting of human intervention studies. Written informed consent will be obtained from all eligible patients before enrollment.

Consent for publication

The author have obtained consent from the participants to publish individual patient data.

Availability of data and material

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Authors’ contributions

The protocol was developed by LP-H, XL-P, JZ and DZ helped with participants’ recruitment and management. LP-H, XL-P, JZ and DZ contributed to study conception and design, and project planning. NW and ZW-Z contributed to laboratory assays, and CY-W provided advice on the statistical analysis. LP-H, XL-P and JZ draft and revised the initial manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Schedule for screening, intervention and follow up (test visit)

|                                | Screening (-4 week) | Test visit 1 (week 0) | Test visit 2 (week 6) | Test (week) |
|--------------------------------|---------------------|-----------------------|-----------------------|-------------|
| Screening questionnaire         | X                   |                       |                       |             |
| Demographic data               | X                   |                       |                       |             |
| Medication, health status and any changes | X   | X                     | X                     | X           |
| Informed consent               | X                   |                       |                       |             |
| Height and weight              | X                   | X                     | X                     |             |
| Waist circumference and hip circumference | X   | X                     | X                     |             |
| Body composition (bioelectric impedance) | X   | X                     | X                     |             |
| Blood pressure                 | X                   | X                     | X                     |             |
| Blood sample                   | X                   | X                     | X                     |             |
| Allocation                     | X                   |                       |                       |             |
| Physical activity questionnaire | X                   |                       |                       |             |
| Mini-mental State              | X                   |                       |                       |             |
| 24-h diet recall               | X                   |                       |                       |             |
| Spot urine sample              | X                   | X                     |                       |             |
| Fecal sample                   | X                   |                       |                       |             |
| Given supply of capsules       | X                   |                       |                       |             |
| Table count                    | X                   |                       |                       |             |
| Compliance assessment          | X                   |                       |                       |             |
| Assess adverse reactions       | X                   |                       |                       |             |
| Collect unused test product    | X                   |                       |                       |             |

Table 2. Biological and anthropometric parameter analysed in the lutein supplementation study
| Variable* | Screeing (-4 week) | Test visit 1 (week 0) | Intermediate visit (week 6) | Test visit 2 (week 12) |
|-----------|--------------------|----------------------|-----------------------------|------------------------|
| **Biological** | TC | X | X | X | X |
| | TG | X | X | X | X |
| | HDL-c | X | X | X | X |
| | LDL-c | X | X | X | X |
| | ALT | X | X | X | X |
| | AST | X | X | X | X |
| | Creatinine | X | X | X | X |
| | FBG | X | X | X | X |
| | Insulin | X | X | X | X |
| | Lutein | X | X | X | X |
| | RBP4 | X | X | X | X |
| | Adiponectin | X | X | X | X |
| | IL-6 | X | X | X | X |
| | IL-1β | X | X | X | X |
| | CRP | X | X | X | X |
| | MCP-1 | X | X | X | X |
| | TNF-α | X | X | X | X |
| **Anthropometric measurement** | WC | X | X | X | X |
| | HC | X | X | X | X |
| | BMI | X | X | X | X |
| | WHR | X | X | X | X |
| | WHtR | X | X | X | X |
| | BP | X | X | X | X |

* ALT: alanine aminotransferase; AST: aspartate aminotransferase; BP: blood pressure; BMI: body weight mass; CRP: C-reactive protein; FBG: fasting blood glucose; HC: hip circumference; HDL-C: high-density lipoprotein cholesterol; IL-6: interleukin-6; IL-1β: Interleukin-1β; LDL-C: low-density lipoprotein cholesterol; MCP-1: monocyte chemoattractant protein-1; RBP4: retinol-binding protein 4; TC: total cholesterol; TG: triglyceride; TNF-α: tumor necrosis factor-α; WC: waist circumference; WHtR: waist-to-height ratio; WHR: waist-to-hip ratio;

**Additional File**

**Additional file 1:** SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents.

**Figures**
Recruitment for volunteers at the community and arrange screening visit when volunteer is willing to take part in the study

Excluded (n=): not meeting inclusion criteria

Screening visit (visit 0)
1. Give overview of the study procedures to volunteer
2. Further assess the potential eligibility of the participants
3. Consent from sign by research and volunteer
4. Book first study visit and give instruction for the first study visit

Excluded (n=): not meeting inclusion criteria (n=)
refuse to participate or other reason (n=)

Randomization (n=120)
1. Subjects stratification according to gender, age and waist circumstance
2. Randomly assigned into the intervention group and placebo group at 1:1 ratio
3. Allocation concealment will be implemented by using the black envelope

Lutein group (n=60)
Placebo group (n=60)

Follow-up
Lost to follow up (n=)
Discontinued intervention (n=)
Reason(s): Declined to participate (n=)
Others (n=)

Lost to follow up (n=)
Discontinued intervention (n=)
Reason(s): Declined to participate (n=)
Others (n=)

Analysis (n=)
Excluded from the analysis (n=)

Analysis (n=)
Excluded from the analysis (n=)

Figure 1
Flow chart of the trial

Supplementary Files
This is a list of supplementary files associated with the primary manuscript. Click to download.
Additional file 1.pdf
