Effects of intermittent caloric restriction in youth with cardiometabolic risk: a randomized controlled pilot study

Statistical Analysis Plan

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1. Introduction

The purpose of this Statistical Analysis Plan (SAP) is to define the outcome variables, statistical methods, and analysis strategies to address the objectives in the randomized, controlled pilot trial for effects of intermittent caloric restriction in youth with cardiometabolic risk.

This study is a randomized controlled pilot trial of intermittent caloric restriction versus low carbohydrate diet in youth with cardiometabolic risk over one month period. The experimental intervention is based on the 5:2 diet, which involves caloric restriction for 2 days (consecutive or nonconsecutive, 600 kcal/d for male and 500 kcal/d for female) per week and unrestricted eating during the other 5 days of the week. For control group, the subjects receive 7 days (consecutive or nonconsecutive) of low carbohydrate diet intervention during the 14-day intervention period. Carbohydrate intake of low-carbohydrate diet should be controlled as ≤ 50 g per day. Total trial duration is one month consisting of a 14-day intervention phase and a 14-day self-maintenance phase. Follow-up electronic questionnaires were conducted during the maintenance phase.

2. Study objectives and outcomes

2.1. Study objectives

2.1.1. Primary objective

The primary objective is to evaluate the reversal of cardiometabolic abnormality between intermittent caloric restriction and low carbohydrate diet in youth after 14-day intervention phase.

2.1.2. Secondary objectives

To make these comparisons between the two groups from the baseline (the first day of the intervention phase) to the 14th day of the intervention phase or the 14th day of the self-maintenance phase:

(1) Change in insulin.

(2) Change in insulin-like growth factor-1.

(3) Change in mean blood glucose.

(4) Change in gut microbial compositions.

(5) Change in body weight.

(6) Change in waist circumference.

2.2. Outcomes

2.2.1. Primary outcome

Reversal (%) of cardiometabolic abnormality.

The primary outcome is a composite outcome that will be measured as any reverse ~~outcome~~ of the following cardiometabolic abnormality: (1) overweight or obesity, (2) prediabetes, (3) hyperlipidemia or (4) elevated blood pressure. It is defined as occurring if any cardiometabolic abnormality of individual subject has a reversal outcome after 14 days dietary intervention (measured at the 14^{th} day).

The primary endpoint will be summarised by number (%) of subjects that with at least one reverse of cardiometabolic abnormalities.

Reversal of cardiometabolic abnormality is defined as:

At the 14th day of dietary intervention phase, at least one of the following indicators changed from abnormal at baseline to normal: overweight, obesity, prediabetes, hyperlipidemia and elevated blood pressure.

(The reversal of overweight or obesity is defined according to references [1, 2]:

For subject with general obesity at baseline: weight loss of 1.5 kg after intervention;

For subject with central obesity at baseline: waist circumference reduction of 1.5 cm after intervention.)

Time Frame:

From the baseline (the first day of the intervention phase) to the 14th day of the intervention phase.

2.2.2. Secondary outcomes

- Change in insulin measurements from fasting blood sample. (Time Frame: from the baseline (the first day of the intervention phase) to the 14th day of the intervention phase.)
- Change in insulin-like growth factor-1 from fasting blood sample. (Time Frame: from the baseline to the 14th day of the intervention phase.)
- Change in mean blood glucose. (Time Frame: from the baseline to the 14th day of the intervention phase.)
- Change in gut microbial compositions. (Time Frame: from the baseline to the 14th day of the intervention phase.)
- Change in body weight (kg). (Time Frame: from the baseline to the 14th day of the intervention phase.)
- Change in body weight (kg). (Time Frame: from the baseline to the 14th day of the self-maintenance phase.)
- Change in waist circumference (cm). (Time Frame: from the baseline to the 14th day of the intervention phase.)

- Change in waist circumference (cm). (Time Frame: from the baseline to the 14th day of the self-maintenance phase.)

2.2.3. Case ascertainment and case definitions

(1) Cardiometabolic abnormalities

① Overweight or obesity (central obesity or general obesity)

② Prediabetes: impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT)

③ Dyslipidemia

④ Elevated blood pressure

Definitions for cardiometabolic abnormalities:

① Overweight or obesity

➢ For subjects ($9 \leq$ age ≤ 18 years) :

• General obesity: Body mass index higher than the 85th percentile for overweight and the 90th percentile for obesity, based on the references for screening overweight and obesity in Chinese children and adolescents.

• Central obesity: Waist circumference higher than the 90th percentile of the age and gender-specific reference for screening cardiovascular risk factors in Chinese children and adolescents.

➢ For subjects ($19 \leq$ age ≤ 30 years) :

• General obesity: Body mass index between 24.0 and 27.9 kg/m2 for

overweight and ≥28 kg/m2 for obesity.

• Central obesity: Waist circumference ≥85cm for men and ≥80cm for female. Based on recommendation of overweight and obesity in Chinese adults.

② Prediabetes:

With IFG and/or IGT. IFG: fasting glucose from 5.6 to 6.9 mmol/L; IGT: 2-h glucose in the 75g oral glucose tolerance test from 7.8 to 11.0 mmol/L. Based on recommendation of American Diabetes Association.

③ Dyslipidemia:

- For subjects (9 ≤ age ≤ 18 years) :

Triglycerides of ≥1.70 mmol/L or total cholesterol of ≥5.18 mmol/L or lowdensity lipoprotein cholesterol ≥3.37 mmol/L or highdensity lipoprotein cholesterol of ≤1.04 mmol/L. Based on recommendation in Chinese children and adolescents.

- For subjects (19 ≤ age ≤ 30 years) :

Triglycerides of ≥1.7 mmol/L or total cholesterol of ≥5.2 mmol/L or lowdensity lipoprotein cholesterol ≥3.4 mmol/L or highdensity lipoprotein cholesterol of ≤1.0 mmol/L. Based on guideline for the management of dyslipidemia in Chinese adults.

④ Elevated blood pressure:

- For subjects (9 ≤ age ≤ 18 years) :

Blood pressure higher than the 90th percentile of blood pressure age and gender-specific reference standards for Chinese children and adolescents, or blood pressure >120/80 mmHg.

- For subjects (19 ≤ age ≤ 30 years) :

Systolic blood pressure ≥120 mmHg and/or diastolic blood pressure ≥80 mmHg. Based on Chinese guidelines for the management of hypertension.

3. Study Design

3.1. Design

This study is a randomized controlled pilot trial to compare the effects of intermittent caloric restriction versus low carbohydrate diet on cardiometabolic risk factors in youth.

3.2. Trial Sites

The trial is conducted in Shanghai, China.

3.3. Interventions

3.3.1. Experimental arm:

Intermittent caloric restriction

The experimental intervention is based on the 5:2 diet, which involves caloric restriction for 2 days (consecutive or nonconsecutive, 600 kcal/d for male and 500 kcal/d for female) per week and unrestricted eating during the other 5 days of the week. Total trial duration is one month consisting of a 14-day intervention phase and a 14-day self-maintenance phase. During the maintenance phase, two follow-up electronic questionnaires were conducted.

3.3.2. Control arm:

Low carbohydrate diet

During the 14-day intervention period, the subjects receive 7 days (consecutive or nonconsecutive) of low carbohydrate diet intervention. Carbohydrate intake of low-carbohydrate diet should be controlled as $\leqslant$ 50g per day. Total trial duration is one month consisting of a 14-day intervention phase and a 14-day self-maintenance phase. During the maintenance phase, two follow-up electronic questionnaires were conducted.

3.3.3. Additional health care: health education on reducing the cardiometabolic risk

Health education is conducted once a week during 14-day dietary intervention for all subjects. Health education including the understanding of cardiovascular disease, how to determine the cardiometabolic risk level, and the lifestyle intervention as caloric restriction and increased physical activity to promote health.

3.4. Randomisation

Randomisation is stratified by two age groups and with block size of 2 and allocation of 1:1 at each stratum. The randomisation was done by Stata software version 15. For each stratum a sequence of subjects IDs and intervention allocations was generated, which by creating a random variable and ordering on that variable. After signing the informed consent form, the subjects are assigned to the experimental or the control groups and receive the designed intervention.

4. Analysis populations

4.1. Study population data sets

Two study populations will be considered in the analysis as follows:

Intent-to-Treat population

Intent-to-treat (ITT) will be defined at the moment the randomisation is performed. For the ITT analysis in this trial, participants will be followed

with their ITT arms. For example, if a participant do not adhere to interventions, or receives the wrong treatment, they will be analyzed according to their primary allocations.

Per-protocol population

Per protocol population will be defined after excluding participants who met the following situations:

Participants will be excluded from the per-protocol population if they:

- The primary outcome is missing (reversal of cardiometabolic abnormalities);
- Did not adhere to the allocated intervention, or switched intervention.

The treatment groups in the per-protocol analysis will be defined according to what the participant actually received. This population will be used for the supportive analyses.

4.2. Analysis Close Date

The analysis close date is the date on which the last participant completed 2-week follow-up.

4.3. Data cleaning

The data will be checked to ensure that there are no erroneous entries and that all missing data is properly coded. Any changes will be made on the ACCESS database.

4.4. Data download

Once all data have been inputted and checked, the database will be locked and a data download request made. The data will be downloaded into STATA formats for statistical analyses.

5. Statistical Analyses

5.1. Primary Outcome Analysis

5.1.1. ITT analysis of the primary outcome - the primary analysis

The primary outcome is a binary composite outcome: occurrence of reversal outcome from any cardiometabolic abnormality at the 14th day after dietary intervention phase. The primary analysis will be based on the ITT population as defined above.

The primary endpoint will be summarised by number (%) of subjects that have reverse cardiometabolic abnormality by groups. Statistical analysis will be performed as a two-sided test, using chi-square test for detecting group difference of the percentage. If any subjects dropped off from the

primary endpoint, modified ITT analysis will be performed by using the available subjects.

A generalised linear model (GLM) will be used. In the GLM model, the occurrence of reversal outcome from any cardiometabolic abnormality at the 14th day after intervention phase will be treated as the response variable following a binomial distribution, the treatment as fixed effect and logit link function will be used. From this model, the reversal rate difference and its 95% CIs between two groups will be estimated.

Covariate adjusted analysis of the primary outcome

An analysis of the primary endpoint will be adjusted for age, sex, family history of cardiovascular disease, and fasting insulin concentrations at baseline by GLM model. The model may not converge when all covariates are introduced into the model simultaneously. If this occurs, a covariate will be removed from model one by one starting from the last covariate until the model converges.

5.1.2. Subgroup analysis of the primary outcome

Subgroup analyses will be performed on age groups. Age will be categorised at ≤ 18 or more. Comparisons between groups for reversal rate after the 14-days intervention period will be analysed.

5.2. Secondary Outcome Analysis

All secondary outcomes will be analysed and two-sided 95% CIs for the treatment differences in these outcomes between the two arms will be calculated and presented. Secondary outcome analyses will be based on the ITT population unless specified.

5.2.1. Analysis of binary outcomes

Binary secondary outcomes will be analyzed with the same strategy and method with that is used for the primary outcome.

5.2.2. Analysis of continuous outcomes

The continuous outcome will be summarised using number of subjects (n), mean, standard deviation (SD), minimum, and maximum by intervention group, and will be analysed by a GLM model with treatment as fixed effect and with normal distribution and identity link function. Difference in mean outcome and mean differences with their two-sided 95% confidence intervals between two groups will be derived from the GLM model.

6. General considerations for data analyses

STATA® (version 15.0) will be used to perform all data analyses and generate the majority of data displays.

6.1. Data Summaries

Continuous variables will be summarized according to number of subjects

with non-missing data (n), mean, standard deviation (SD), median, minimum, and maximum. The confidence intervals will be reported on summaries of continuous effectiveness variables.

Categorical variables will be summarized according to the absolute frequency and percentage of subjects (%) in each category level. The denominator for the percentages is the number of subjects in the treatment arm with data available, unless noted otherwise.

6.2. Graphical Displays

Mean values for some continuous outcomes by treatment will be plotted.

7. References

[1] Harvie MN, Pegington M, Mattson MP, et al. The effects of intermittent or continuous energy restriction on weight loss and metabolic disease risk markers: a randomized trial in young overweight women. Int J Obes (Lond). 2011 May;35(5):714-27.

[2] Arnason TG, Bowen MW, Mansell KD. Effects of intermittent fasting on health markers in those with type 2 diabetes: A pilot study. World J Diabetes. 2017 Apr 15;8(4):154-164.

8. Study variable list

NO	**Variables**	
	Baseline	
1	Name	T
2	Sex	B
3	Recruited date	D
4	Birth date	D
5	Height	N
6	Weight	N
7	Waist circumstance	N
8	Age	N
9	Family history of cardiovascular disease	B
10	Fasting blood glucose (mmol/L)	N
11	OGTT 2-hours glucose (mmol/L)	N
12	Glycosylated hemoglobin	N
13	Fasting insulin (mIU/L)	N
14	Total cholesterol (mmol/L)	N
15	Triglyceride (mmol/L)	N
16	High density lipoprotein (mmol/L)	N
17	Low density lipoprotein (mmol/L)	N
18	Systolic pressure (mmHg)	N
19	Diastolic pressure (mmHg)	N
20	Insulin-like growth factor-1	N
21	Gut microbial compositions	N
	Dietary intake	
22	Record date *(during 14-days intervention period)*	D
23	Energy (kcal/d)	N
24	Protein (g/d)	N
25	Fat (g/d)	N
26	Carbohydrate (g/d)	N
	Continuous glucose monitoring	
27	Record date *(during 14-days intervention period)*	D
28	24-hour average blood glucose	N
	At the 14th day after the dietary intervention phase	
29	Weight	N
30	Waist circumstance	N
31	Fasting blood glucose (mmol/L)	N
32	OGTT 2-hours glucose (mmol/L)	N
33	Fasting insulin (mIU/L)	N
34	Total cholesterol (mmol/L)	N
35	Triglyceride (mmol/L)	N

NO	**Variables**	
36	High density lipoprotein (mmol/L)	N
37	Low density lipoprotein (mmol/L)	N
38	Systolic pressure (mmHg)	N
39	Diastolic pressure (mmHg)	N
40	Insulin-like growth factor-1	N
41	Gut microbial compositions	N
	At the 14th day after the self-maintenance phase	
42	Weight	N
43	Waist circumstance	N
44	Self-reported dietary adherence	B
45	Psychological and symptoms during dietary intervention	B

Note: T, text variable; D, The date type; N, Continuous variable; B, binary variable.