Combined Effects of Vitamin D Supplementation and Endurance Exercise Training on Insulin Resistance in Newly Diagnosed type 2 Diabetes Mellitus Patients with Vitamin D Deficiency : Study Protocol for a Randomized Controlled Trial

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Study protocol

Keywords: Supplementation, Endurance, Insulin Resistance, Mellitus Patients

Posted Date: June 14th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-42821/v1

Version of Record: A version of this preprint was published at Trials on December 1st, 2021. See the published version at https://doi.org/10.1186/s13063-021-05861-x.

Abstract

Background

Although approximately 50% of Chinese T2DM patients have vitamin D deficiency, studies regarding vitamin D supplementation on IR have mainly focused on non-Asians. Endurance exercise training (ET) enhances insulin-mediated glucose metabolism, which plays a critical role in T2DM prevention and control. However, the combined effects of vitamin D supplementation and ET on IR in T2DM patients are unclear.

Methods and analysis

We propose a randomized controlled trial among 60 T2DM patients with vitamin D deficiency to evaluate the combined effects of vitamin D supplementation and endurance training on IR. The participants will be randomly allocated to the vitamin D group, vitamin D combined with exercise training group, exercise training group, and control group (CG) using a computer-generated random number sequence. At baseline, participants will undergo a medical review, anthropometric measurements, dual X-ray absorptiometry, a 75-g oral glucose tolerance test, ankle-brachial index measurements, and physical fitness measurements and will complete related lifestyle questionnaires. In a 3-month intervention period, vitamin D intervention group will receive a dose of 1000IU daily; exercise group will perform a 1-hour endurance exercise 3 times per week (maximal heart rate, 60%–80%), and the control group will receive apparently identical tablets. Additionally, all participants will be advised to maintain their normal diet and physical activities during the intervention. All measurements will be repeated at 3-month follow-up after the intervention with the primary outcome measure expressed as a change from baseline in insulin sensitivity and secretion. Secondary outcome measures will compare the changes in anthropometry, ankle-brachial index, and physical fitness factors. Data will be managed and analyzed using the Statistical Package for the Social Sciences.

Discussion

This is the first study to conduct a randomized trial to clearly determine the independent and combined effects of vitamin D supplementation and endurance exercise trial on IR in Chinese T2DM patients as measured by OGTT. The findings from the proposed study will not only provide new evidences that vitamin D supplementation plays an important role in reducing IR but also develop a simple and efficient method to improve IR and associated metabolic diseases for T2DM patients.

Trial registration

Chinese Clinical Trial Registry, ChiCTR1800015383, Registered 28 March 2018, http://www.chictr.org.cn

Introduction

With rapid social and economic developments during the past three decades, China is facing a growing threat from non-communicable chronic diseases (NCDs), with diabetes being considered one of the most common NCDs in China and several countries. [1] Diabetes prevalence in Chinese adults increased substantially from 0.67% in 1980 to 11.6% in 2010 and 10.9% in 2013.[2] Insulin resistance (IR) is considered a prominent feature of type 2 diabetes mellitus (T2DM) and an important pathological and physiological basis for the development of T2DM.[3] How to effectively improve IR has become one of the emphases in the treatment of T2DM.

Mounting evidence suggests that altered vitamin D homeostasis may play a role in the development of IR and T2DM.[4] A prospective cohort study and meta-analysis indicated that subjects with low 25-hydroxyvitamin D [25(OH)D] levels were more likely to be diagnosed with T2DM compared to subjects with high 25(OH)D levels.[5] The results were also supported by several vitamin D supplementation studies on IR in Japanese adults and South Asian women with IR.[6,7]

Vitamin D deficiency [25(OH)D<20ng/mL) is prevalent in several populations and has become a common public health problem worldwide due to sun protection measures, reduction of outdoor activities, and environmental pollution.[8] Zhang and colleagues recently reported that in China, 50% of T2DM patients were vitamin D deficient, further deteriorating their glucose tolerance status.[9] However, to date, studies on vitamin D supplementation on IR in T2DM patients have mainly focused on non-Asian countries, and the results of these studies are inconsistent,[10-15] which may be partly attributed to the ethnicity-related differences in vitamin D receptor (VDR) polymorphisms and insulin sensitivity.[16,17] Furthermore, the independent effect of vitamin D on IR is possibly limited in T2DM patients. Krul-Poel et al. conducted a 6-month randomized controlled trial of vitamin D supplementation in 275 T2DM patients and found that glycated hemoglobin (HbA1c) levels did not improve in individuals with vitamin D deficiency and/or insufficient.[10] Similar results were also observed in German T2DM patients.[11]

Documented evidence demonstrated that exercise intervention is one of most effective traditional lifestyle interventions to prevent and treat T2DM.[18] Exercise training not only increases energy consumption and reduces the accumulation of lipids in insulin-sensitive tissue such as the skeletal muscle[19] but also promotes glucose transportor-4 (GLUT-4) translocation and glucose uptake in skeletal muscle cells by activating AMP-activated protein kinase (AMPK) and downstream proteins of insulin-transmitting signals in skeletal muscle cells.[20] Additionally, recent studies have shown that exercise may also be involved in the regulation of vitamin D, which can increase VDR expression in skeletal muscle tissue and serum 25(OH)D levels.[21-23] Prior evidence suggested that in addition to regulating Ca^{2+} levels, vitamin D supplementation can directly upregulate the AMPK-GLUT-4 signaling pathway through VDR to increase glucose utilization and participate in exercise pathways of glucose utilization pathways. These results suggested that vitamin D supplementation combined with exercise intervention may have a synergistic effect on the improvement of IR by activating different glucose utilization pathways. However, studies investigating the synergistic effect of vitamin D supplementation combined with exercise training intervention on IR in T2DM patients are relatively few.

In summary, this study aimed to (1) examine the effect of vitamin D supplementation on IR and (2) determine the synergistic effect of vitamin D supplementation combined with exercise training intervention on IR in Chinese T2DM patients with vitamin D deficiency by a randomized controlled trial.

Methods And Analysis

Study design

This protocol describes the setting of a 3-month intervention and 3-month follow-up period assessing the impact of combined vitamin D and exercise intervention on improving glucose and lipid metabolism in T2DM patients in Xi'an, China (34°N latitude). Sixty T2DM patients aged 40–65 years with serum 25(OH)D concentrations <20 ng/mL on screening will be enrolled during winter and spring. Patients will be randomly assigned to either an intervention or a control group. The complete process is outlined in Figure 1.

Patient and public involvement

Patients will not be involved in recruitment of participants or conduct of the study. Participant burden of the intervention and measures was assessed by interviews and feedback from patients who participated in previous pilot trail. The intervention content of this study was based on the guideline of the American College of Sports Medicine and Institute of Medicine, and measures were conducted in hospital by specialist doctors and trainers. Finally, the results of this study will feedback and disseminate to all of participants by volunteers and the researchers.

Setting and participants

Inclusion and exclusion criteria

T2DM patients are eligible for study participation if they meet the following inclusion criteria:

(a) patients diagnosed with T2DM ≤1 year, (b) patients with HbA1c level remaining stable at ≤8.0% in the past 3 months and patients with no plan of replacing the hypoglycemic agent in the near future, (c) patients without regular vitamin D and/or calcium supplements in the past year, (d) patients who do not meet the current National Physical Activity guidelines, and (e) patients without regular exercise habits in the past year.

The following participants were excluded: participants with acute infection; participants experiencing stress; participants experiencing the acute complications of diabetes; participants with heart, liver, and kidney insufficiency, osteoporosis and fracture, and metal implants in the body that could affect magnetic resonance imaging and dual-energy X-ray absorptiometry (DXA) measurements; and participants who have used insulin therapy and have history of sunlight exposure recently.

The criteria for early termination of intervention are as follows: (a) blood calcium level is abnormally high (>2.65 nmol/L), (b) serum 25(OH)D levels are abnormally high (>250 nmol/L), and (c) have plan to replace with stronger blood glucose lowering drugs or HbA1c levels are >8.5%.

Randomization

An investigator who is independent of this study will randomly divide the subjects into four groups according to a computer-generated random allocation table stratified by age and sex. To ensure the randomized, double-blind effect of the trial, trial designers, testers, and data collectors are not informed of the vitamin D/placebo intake grouping until the intervention trial and data collection are completely completed. The four groups are as follows: the placebo control group (CG), vitamin D supplementation group (VG), endurance exercise group (EG), and vitamin D supplementation combined endurance exercise group (VEG). All patients will be instructed not to undertake any formal exercise or change their levels of general physical activity and dietary habits during the intervention period.

Intervention procedure

Vitamin D intervention: Patients in the VG or VEG group will receive one tablet of vitamin D_3 supplement (1000 IU/day) immediately after meals, and patients in the EG or CG group will receive an identical appearance shape and color placebo as the vitamin D_3 supplement every day for 3 months.
Exercise intervention: Patients in the EG or VEG group will perform a 1-hour progressively increasing aerobic exercise (cycling, running, or rowing) at 60%–80% of maximal heart rate 3 times a week for 3 months, and this will be supervised by a qualified trainer who is knowledgeable of the study protocol and procedures. Polar monitor will be used to monitor heart rate during exercise, and the compliance with each protocol will be recorded. Participants will warm up during the first 5 min on a

treadmill at 50%–60% of maximal heart rate and subsequently follow the exercise protocol assigned to them, with a 5–10-min recovery exercise at 40%–50% of maximal heart rate, which comprises walking and stretching exercise.

Data collection

Outcome measures and measurement procedures

A full list of measurable outcomes is presented in Table 1. Participants will be assessed at baseline, at the end of intervention, and 3 months after the intervention.

Table1. Summary of outcome measures

Outcomes	Item	Device
Primary outcomes		
Insulin sensitivity and insulin secretion	The Matsuda index, the insulinogenic index, HOMA-IR, HOMA-β	OGTT
		Fasting blood measurements
Vitamin D level	Serum 25(OH)D, $1,25(OH)_2D$	Fasting blood measurements
Secondary outcomes		
Body composition	Percent body fat, muscle mass	DXA
	Waist circumference	Standard tape
Metabolic indices	Fasting glucose (G_0), fasting insulin (I_0), HbA1c, triglyceride, cholesterol, low-density lipoprotein, and high-density lipoprotein	Fasting blood measurements
Ankle-brachial index	Blood pressure, ABI, baPWV	Validated automatic device
Physical fitness factors	Peak oxygen uptake, maximal heart rate	Cycle ergometer
	Hand grip strength	Hand grip dynamometer

HOMA, homeostatic model assessment; IR, insulin resistance; 25(OH)D, 25-hydroxyvitamin D; $1,25(OH)_2D$, 1,25-Dihydroxyvitamin D; OGTT, Oral glucose tolerance test; DXA, dual energy X-ray absorptiometry; HbA1c, glycated hemoglobin; ABI Ankle-brachial index

Analysis of blood samples: A standard 75-g oral glucose tolerance test (OGTT) will be performed between 0830 and 1100 after a 12-hour overnight fast, and venous blood samples will be collected in Venoject-II AutoSep tubes at time points 0, 30, 60, 90, and 120 min to determine the plasma glucose and serum insulin levels. Fasting serum sample will be used to measure the levels of 25(OH)D, $1,25(OH)_2D$, calcium, fasting glucose (G_0), fasting insulin (I_0), HbA1c, triglyceride, cholesterol, low-density lipoprotein, and high-density lipoprotein.

Primary outcomes: changes in insulin sensitivity and secretion

Fasting blood glucose and insulin will be used to calculate the insulin resistance index (Homeostatic Model Assessment of Insulin Resistance [HOMA-IR]) and β-cell secretion index as follows: HOMA-IR = $G_0 \times I_0/22.5$ and HOMA-β = $20 \times I_0/(G_0-3.5)$.

Insulin sensitivity estimated using the Matsuda index of insulin sensitivity during the OGTT will be calculated as follows: 1000/square root of ([G_0×I_0]) × (mean OGTT glucose concentration × mean OGTT insulin concentration). [24] Early phase of insulin secretion will be estimated using the insulinogenic index as follows: $I_{30}-I_0/G_{30}-G_0$ ($\triangle$I30/$\triangle$G30), where I_{30} and G_{30} represent insulin and glucose values at 30 min during the OGTT, respectively.[25] The increments in the area under the curves during the complete 120-min period of the OGTT will be calculated using the trapezoid rule to assess the total changes in glucose and insulin levels.

Secondary outcomes: changes in anthropometry, ankle-brachial index, and physical fitness factors

Anthropometry

Height and body mass will be measured with the participants in light clothing and barefoot. Body mass index will be calculated by dividing the body mass in kilograms by the square of height in meters (kg/m^2). Waist circumference will be measured to the nearest 0.1 cm at the umbilical region using an inelastic measuring tape at the end of normal expiration. DXA is used to measure percent body fat and muscle mass (Hologic QDR-4500, DXA Scanner, Hologic Inc., Waltham, MA, USA) by a recognized technologist.

Ankle-brachial index

Blood pressure and brachial-ankle pulse wave velocity (baPWV) will be measured after the participant had rested supine for at least 5 min using a validated automatic device (BP-203RPE, II form PWV/ABI, Omron-Colin, Japan) in the brachial and ankle arteries. An oscillometric method will be used, and its cuffs have sensors that transmit data to the device. The baPWV will be calculated by dividing the distance from the aortic valve to the ankle artery by the sum of the time between the aortic valve closing sound and the notch of the brachial pulse wave and the time between the increase in the brachial pulse wave and that of the ankle pulse wave. The ankle-brachial index will be calculated by dividing the highest value obtained at each ankle by the highest of the arm values.

Physical fitness factors

Peak oxygen uptake (VO_{2peak}) will be measured using a maximal graded exercise test using a cycle ergometer (MetaMax 3B, Cortex, Germany). The graded cycle exercise will begin at a workload of 45–90 W, which is subsequently increased by 15 W/min until the subject could not maintain the required pedaling frequency of 60 rpm. During the progressive exercise test, the expired gas of subjects will be collected, and the rates of oxygen consumption and carbon dioxide production will be measured and averaged over 30-s intervals using an automated gas analyzing system. The highest recorded value of VO_2 and heart rate during the exercise test will be quantified as the VO_{2peak} (mL·kg-1·min-1) and maximal heart rate (bpm). Vigorous activities and alcohol and caffeinated beverages will be prohibited 2 days before the test.
Handgrip strength will be measured using a hand grip dynamometer (HK6800-WL, Shenzhen, China) in

units of kilograms. Participants will be instructed to complete two handgrip contraction trials bilaterally, alternating hands between trials. The highest values obtained using each hand were considered the right-hand and left-hand grip strength scores. The mean value of the two maximal grip strength scores will be used.

Sunlight exposure

Participants will be instructed to record their outdoor activity time and exposed areas of the skin from 9 am to 5 pm for 7 consecutive days in a week using a questionnaire every month. A score to estimate the mean weekly sunlight exposure will be calculated, and additional details were published elsewhere.[26]

International Physical Activity Questionnaire

Daily physical activity except for the exercise program will be assessed using the International Physical Activity Questionnaire monthly and expressed as metabolic equivalent minutes per week. It refers to the preceding 7 days and instructs participants to report the number of days, hours, and minutes spent on vigorous activity, such as aerobics, or moderate activity, such as carrying light loads.[27]

Follow-up visits

Participants will be scheduled for their follow-up visits during the last week of their treatment assessing all procedures performed during baseline assessment including blood pressure measurement, anthropometry, OGTT, DXA, and physical activity and dietary assessment using structured questionnaires.

Safety considerations

The dose of vitamin D used in the present study is expected to significantly increase the serum 25(OH)D concentrations and decrease the glucose levels in T2DM patients[28] and is lower than the tolerable upper dose for Chinese.[29] During the screening, baseline, and follow-up, any medical conditions or abnormalities detected will be promptly discussed with the participant by a qualified medical practitioner involved in the study. Participants are allowed to be treated, referred, or advised to visit their practitioner during the intervention. All participants will be informed of their screening blood test results after they have completed their participation in the study.

Statistical analyses

Based on the relative studies on vitamin D intervention with IR,[6] the sample size required for each group was estimated to be 12 with a power of 85%, and an effect size of 0.26 was assumed. Therefore, 48 T2DM patients are required. To account for 20% loss-to-follow-up, a total of 60 T2DM patients will be recruited. Power calculations will be performed using G*Power software version 3.1.9.2. [30]

Descriptive statistics will be calculated using mean (standard deviation) for continuous variables and n (%) for categorical variables. Differences will be compared using one-way analysis of variance (ANOVA)

for continuous variables and chi-squared tests for categorical variables. Repeated measures ANOVA will be used to test the effect of vitamin D and exercise combined intervention on glucose and lipid metabolism, with adjustment for baseline levels of outcomes. A post hoc test with Bonferroni correction will be used to identify significant differences among the mean values when a significant main effect or interaction is identified. Finally, in the event of possible losses or dropouts, a statistical analysis will be performed by protocol and intention to treat. Data will be entered and cleaned using EpiData version 3.0 and managed and analyzed using the Statistical Package for the Social Sciences. Statistical significance will be set at $P < 0.05$.

Discussion

To the best of our knowledge, this is the first study to conduct a randomized trial to clearly determine the independent and combined effects of vitamin D supplementation and endurance exercise trial on IR in Chinese T2DM patients as measured by OGTT. The findings from the proposed study will not only provide new evidences that vitamin D supplementation plays an important role in reducing IR but also develop a simple and efficient method to improve IR and associated metabolic diseases for T2DM patients.

The number of people with T2DM worldwide has increased rapidly. It is estimated to reach over 700 million in 2045 including more than 120 million people with T2DM from China,[31] which can lead to major social, health, and economic challenges. IR is not only considered an important pathological and physiological basis for the development of T2DM but also a major contributor to other complications, which would lead to atherosclerosis, myocardial infarction, stroke, and even death.[32] Hence, the effective and innovative methods of preventing and improving IR for T2DM population are urgently required.

Beyond its traditional role in maintaining bone health, higher serum vitamin D level has also been found to be associated with low risk of developing T2DM and other metabolic diseases considering its potential effects on IR.[3, 33,34] However, to date, the results of several studies conducted are inconsistent.[10-15] Moreover, considering the ethnic differences in insulin metabolism[35] and that studies on vitamin D supplementation on IR in T2DM patients have mainly focused on non-Asian populations, it is significantly important to investigate the effects of vitamin D on IR in Asians.

In addition to promoting GLUT-4 translocation and glucose uptake in skeletal muscle cells,[19, 20] exercise training may also be involved in the regulation of vitamin D through increasing VDR expression in skeletal muscle tissue and serum 25(OH)D levels.[22, 23, 36] Meanwhile, vitamin D supplementation can directly upregulate AMPK-GLUT-4 signaling pathway through VDR to increase glucose utilization and participate in exercise pathway of glucose utilization.[37] Therefore, the combined intervention of vitamin D and exercise on IR for T2DM patients in this study is scientific and novel, and findings of their combined effect from activating different ways of glucose utilization are expected to benefit both T2DM and other chronic metabolic diseases. Finally, we plan to further investigate protein expression related with glucose

uptake in skeletal muscle tissue to elucidate the mechanism of the combined effect on IR in mice in our future study.

China is currently facing a growing threat from NCDs, and it shows no sign of abating. Prevention of NCDs including diabetes through promoting healthy eating and lifestyle has been elevated to a national public policy priority.[2, 38] In October 2016, the State issued the “Healthy China 2030” bringing the NCD issue into sharper and more concrete focus.[39] The findings from this study of vitamin D supplementation on IR for Chinese T2DM patients can also contribute to the alleviation of T2DM epidemic worldwide.

Trial status

Active protocol version number: 1.3; January 1, 2019. The details of the protocol versions with the date of the amendment are provided in Table 2. Recruitment began on April 1, 2018. Currently, recruitment is ongoing and is expected to be completed in December 2020, and intervention and the last follow-up are expected to be completed in December 2021.

Table 2 Protocol versions

Version	Date and changes
1.	March 7, 2017 , original protocol.
1.1	March 1, 2018, introduction and background was improved.
1.2	July 15, 2018, amendments of the inclusion and exclusion criteria.
1.3	January 1, 2019, more comprehensive plan of the analysis was incorporated.

Abbreviations

ABI, Ankle-brachial index; ANOVA, analysis of variance; AMPK, AMP-activated protein kinase; CG, control group; DXA, dual energy X-ray absorptiometry; EG, endurance exercise group; GLUT-4: glucose transportor-4; HbA1c, glycated hemoglobin; HOMA, homeostatic model assessment; IR: Insulin resistance; MHR. Maximal heart rate; OGTT, oral glucose tolerance test; T2DM: type 2 diabetes mellitus; VD, vitamin D; VEG, vitamin D and endurance exercise group; 25(OH)D: 25-hydroxyvitamin D.

Declarations

Ethics approval and consent to participate

This trial has received ethical approval from the Xi’an Jiaotong University Human Research Ethics Committee (protocol ID: 2017446). Informed written consent to participate will be obtained from all participants. We plan to submit the final report of this project to a peer-reviewed journal for publication.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

The present study was supported in part by the National Natural Science Foundation of China [Grant Number: NSFC 81703220]. The funder has no role in the study design, collection, management, analysis, or interpretation of data or writing of this report.

Authors' contributions

All authors contributed to the design and conception of the study. MX, XS and JW drafted the manuscript. XS and ZC contributed to the revision of the manuscript. All authors have read and approved the drafts of the final manuscript.

Acknowledgements

Not applicable.

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Figures

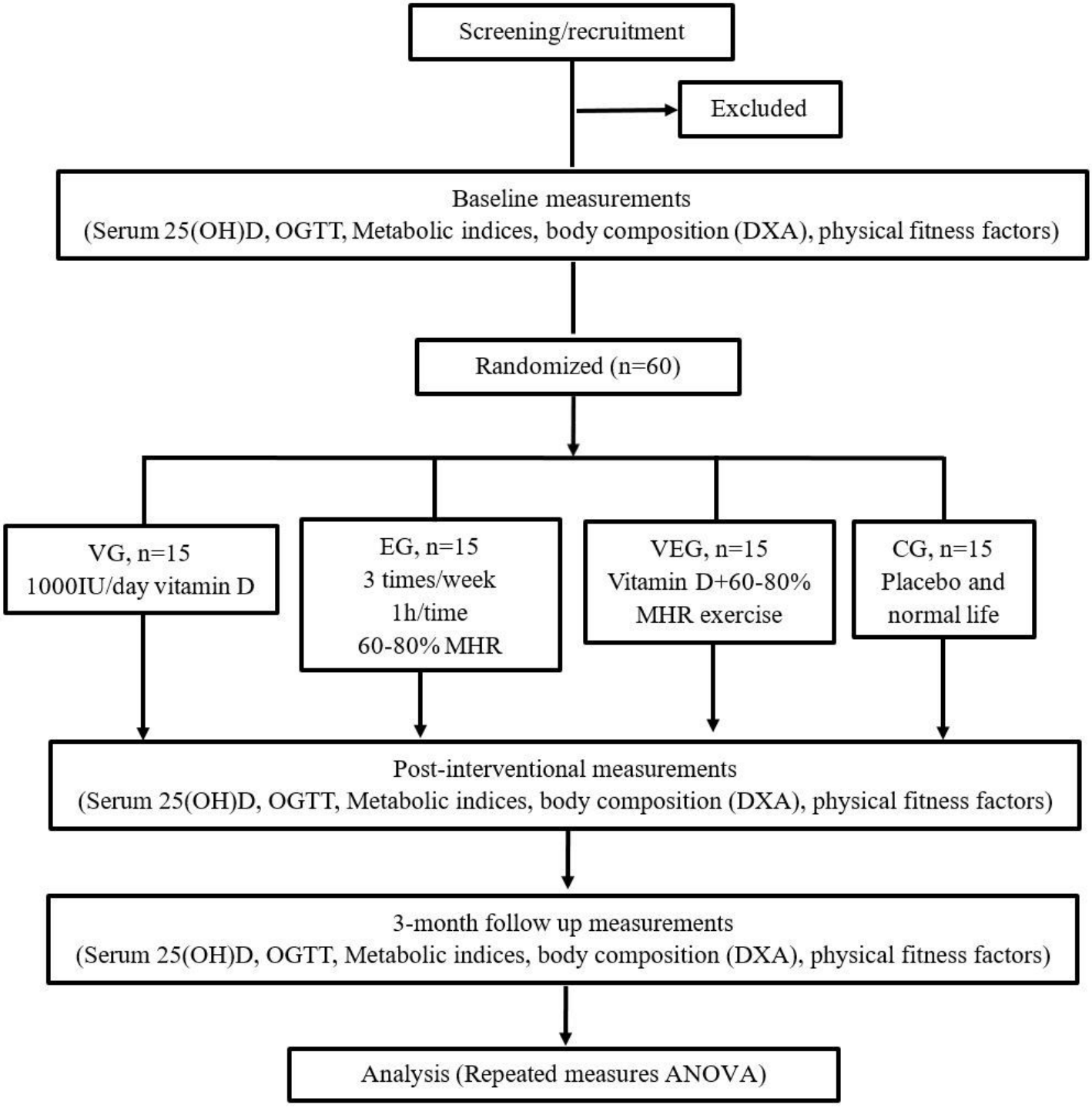

Figure 1

Study design flow chart. OGTT, oral glucose tolerance test; DXA□dual X-ray absorptiometry; VD, vitamin D; EG, endurance exercise group; VEG, vitamin D and endurance exercise group; CG, control group; MHR. Maximal heart rate; ANOVA, analysis of variance.

	STUDY PERIOD					
	Enrolment	Baseline-allocation	Intervention		Follow-up	
	Enrolment	Baseline-allocation	Intervention	Post-Intervention	Follow-up	Close-out
TIMEPOINT**	*-t1*	0	*T2: 1-12weeks*	*T3 13weeks*	*T4 13-24week*	*T5 25weeks*
ENROLMENT:						
Eligibility screen	X					
Informed consent	X					
Randomization and allocation	X					
Baseline-data	X					
INTERVENTIONS:						
[Exercise intervention]			X			
[Vitamin D intervention]			X			
[Vitamin D+ exercise]			X			
ASSESSMENTS:						
[Insulin sensitivity and insulin secretion]		X		X		X
[Vitamin D level]		X		X		X
[Body composition]		X		X		X
[Metabolic indices]		X		X		X
[Ankle-brachial index]		X		X		X
[Physical fitness factors]		X		X		X

Figure 2

Example template of recommended content for the schedule of enrolment, interventions, and assessments.*