ISRCTN36129007 https://doi.org/10.1186/ISRCTN36129007

Vitamin D, chronic complications of type 2 diabetes mellitus and gut microbiota

Submission date	Recruitment status	[X] Prospectively registered
30/06/2023	No longer recruiting	[ ] Protocol
Registration date	**Overall study status**	[ ] Statistical analysis plan
10/07/2023	Ongoing	[ ] Results
Last Edited	**Condition category**	[ ] Individual participant data
16/02/2024	Nutritional, Metabolic, Endocrine	[ ] Record updated in last year

Plain English summary of protocol

Background and study aims
Diabetes mellitus is a life-long condition in which a person is unable to control their blood sugar levels and it contributes to developing some heart, nerves, gut, eyes and kidney-associated diseases. There are two main types of diabetes, type 1 and type 2, with type 2 diabetes (T2DM) being responsible for most cases. Some evidence associates changes in gut microbiota, natural bacteria living in the intestine, with T2DM and metabolic syndrome. In T2DM, body cells become progressively resistant to insulin and, simultaneously, there is an overload of specialised cells in the pancreas called beta-cells (which are responsible for producing the hormone insulin). When beta-cells become tired of overproducing insulin, blood sugar levels become uncontrolled and different medications are used in order to keep their blood sugar levels in a healthy range (glycaemic variability). Some studies have shown that taking vitamin D supplements can help to reduce fluctuations in blood sugar levels. The aim of this study is to find out whether vitamin D supplementation can improve glycaemic variability and reduce complications associated with parameters, such as renal function and values in tests evaluating heart and leg nerves, in patients with T2DM. This study also evaluates the effects of two antidiabetic drugs (metformin and DPP-IV inhibitors) in gut microbiota patterns as well as potential specific taxon-mediated effects.

Who can participate?
Adults with T2DM aged over 30 years old

What does the study involve?
Participants attend an initial study visit at which they have a sample of blood and stool taken to measure their vitamin D levels, and have body measurements taken. Participants then attend another study visit at which they are given vitamin D supplements to take for three months. After three months, information about glycaemic variability, vitamin D levels and diabetes complications are collected, physical examination and blood and stool analysis.

What are the possible benefits and risks of participating?
The benefits of participating are free access to medical and hospital specialized services, image and laboratory exams and vitamin D levels assessments. Risks are associated with adverse effects of vitamin D intake, minimized by the health support offered by the study center.

Where is the study run from?
University Hospital João de Barros Barreto, Federal University of Pará (Brazil)

When is the study starting and how long is it expected to run for?
May 2022 to February 2026

Who is funding the study?
Investigator initiated and funded (Brazil)
Federal University of Pará (UFPA) (Brazil)

Who is the main contact?
Dr João Soares Felício
felicio.bel@terra.com.br

Contact information

Type(s)
Principal investigator

Contact name
Prof João Soares Felício

ORCID ID
https://orcid.org/0000-0003-4002-988X

Contact details
Mundurucus Street, 4487
Belém
Brazil
66073-005
+55 91 3201 6760
felicio.bel@terra.com.br

Type(s)
Scientific

Contact name
Prof João Soares Felício

Contact details
Mundurucus Street, 4487
Belém
Brazil
66073-005
+55 91 32016760
felicio.bel@terra.com.br

Type(s)
Public

Contact name
Ms Priscila Boaventura Barbosa de Figueiredo

ORCID ID
https://orcid.org/0000-0002-6919-2931

Contact details
Mundurucus Street, 4487
Belém
Brazil
66073-005
+55 91 32016760
pribfigueiredo@gmail.com

Additional identifiers

Clinical Trials Information System (CTIS)
Nil known

ClinicalTrials.gov (NCT)
Nil known

Protocol serial number
03VITDDM2

Study information

Scientific Title
Vitamin D, chronic complications of type 2 diabetes mellitus and gut microbiota

Study objectives
Influence of high dose vitamin D supplementation on glycemic and blood pressure variability and control, chronic complications and gut microbiota pattern in type 2 diabetes mellitus

Ethics approval required
Ethics approval required

Ethics approval(s)
approved 18/10/2022, University Hospital João de Barros Barreto ethics committee (Mundurucus Street, 4487. 2nd floor, Belém, 66073-005, Brazil; +55 91 32016754; cephujbb@yahoo.com.br), ref: 88974918.6.0000.0017

Study design
Prospective randomized placebo-controlled study

Primary study design
Interventional

Study type(s)
Quality of life, Treatment, Efficacy

Health condition(s) or problem(s) studied
Type 2 diabetes mellitus (T2DM)

Interventions
All participants attend four scheduled study visits. At the first visit, participants undergo a physical examination, have blood, urine and stool samples taken, 24 hours ambulatory blood pressure monitoring, evaluation of autonomic cardiovascular neuropathy (ACN), retinography, ankle-brachial index and answer two quality of life and one depression assessments. In this same visit, some patients also have a continuous glucose monitoring system (CGMS) performed.

Five days before the second visit patients with CGMS fill out their seven-point self-monitored blood glucose profile. At the second visit, eligible participants are randomized and receive either vitamin D to take at a dose of 10000 IU/day or placebo for 3 consecutive months, in order to achieve serum levels of at least 30 ng/ml. Three months later, participants attend a third study visit at which the initial assessments are repeated.

24-hour ambulatory blood pressure monitoring is also performed by the oscillometric method. It is installed in the morning and withdrawn after 24 hours, and patients are instructed to maintain their usual activities and write them down in a diary, which should include the time and description of each activity performed. The device is programmed to perform a measurement every twenty minutes during the day and thirty minutes at night, and the arithmetic mean of systolic and diastolic blood pressure was established for each hour, during the waking period, during sleep and at 24 hours.

The evaluation of autonomic cardiovascular neuropathy (ACN) is performed in patients according to the following protocol: patients were submitted to a questionnaire, where the following symptoms are investigated: dizziness, visual disturbances or pre-syncope in orthostatism, dyspnea, nausea, sweating and precordial pain during physical activity, diarrhea, fecal incontinence, intestinal constipation, post-eating vomiting, erectile dysfunction or vaginal lubrication, urinary incontinence, polaciuria, urinary urgency, urinary retention, repetitive urinary tract infection, anhydrosis, hyperhidrosis, heat intolerance and gustatory sweating.

Forms of neuropathies secondary to vitamin B12 deficiency, hypothyroidism, alcoholism and chronic renal failure, diagnosis of leprosy or HIV infection are excluded.

Autonomic tests for ACN are performed in the morning after capillary glycemia, whose value should be between 70 and 250 mg/dL. Patients are instructed to stop drinking alcohol and caffeine, in addition to smoking cessation for at least 8 hours before the test; not to perform vigorous physical exercise in the 24 hours prior to the exam, and if they present with fever (Tax ≥ 37.8) in the last two days, major emotional stress on the previous day or hypoglycemia in the 8 hours prior to the exam, the patients are instructed to reschedule autonomic tests.

Heart rate variability (HRV) is evaluated by a computerized system (VNS-MICRO) through seven parameters (the four Ewing tests and the three bands of the spectral analysis). The Valsalva test is performed with the patient in dorsal decubitus (DD) at 30 degrees during 15 minutes of rest, and after this period, the patient performed respiratory effort to maintain a pressure of 40 mmHg for 15 seconds. At the 14th second, there is a maximum physiological tachycardia. After this effort, the sphygmomanometer valve is released and an electrocardiogram (ECG) is performed for 30 to 45 seconds when a maximum physiologic bradycardia occurred. The reason for Valsalva is the relationship between tachycardia and bradycardia or between the longest and the shortest RR interval.

The orthostatic test (30:15 ratio) is performed by ECG performed in DD under the same conditions above and, after standing up, the relationship between heart rate (HR) or RR intervals corresponding to maximum tachycardia around 15° And the maximum bradycardia around the 30th beat.

The deep breath test (E: I ratio) follows the following protocol: The ECG is performed during a deep breath and expiration lasting at least 5 seconds (each). The E: I ratio is obtained by dividing the maximum HR (inspiration) by the minimum HR (expiration) or the longest RR (E) by the shortest RR (I).

In the orthostatic hypotension (HO) test, the patient remained in DD at 30 degrees for 15 minutes. BP is measured at baseline, one and three minutes after orthostasis. A drop greater than or equal to 20 mmHg in systolic BP was considered altered.

In the study of the HRV by spectral analysis in the three bands (FMB, FB and FA), the patient is in DD at 30 degrees and with spontaneous breathing. An electrocardiographic computerized record is made for 300 seconds. The ECG is analyzed by a mathematical algorithm and expressed in an oscillation amplitude diagram (HR fluctuations per second) versus HR (hertz). The total amplitude of the HRV spectrum consists of three bands:
1. Component of very low frequencies or FMB (0.01-0.04 Hz) that is related to the fluctuations of vasomotor tone connected to thermoregulation and sweating (sympathetic control)
2. Low-frequency components or FB (0.04 to 0.15 Hz) associated with the baroreceptor reflex (sympathetic control with vagal modulation)
3. Components of high frequencies or FA (0.15 to 0.5 Hz), related to the parasympathetic (vagus nerve) control.

Diabetes kidney disease is also evaluated in patients, and to do this, patients are classified according to results in normoalbuminuria (<30 mg/g creatinine), microalbuminuria (≥30 mg/g creatinine and <300 mg/g creatinine), and macroalbuminuria (≥300 mg/g creatinine). Retinopathy is also evaluated through retinographies performed at the first and last visits to patients.

Intervention Type
Supplement

Primary outcome(s)
Glycemic variability measured using a continuous glucose monitoring system (CGMS) from baseline to three months

Key secondary outcome(s))
1. Vitamin D (25(OH)D) levels measured using an immonoassay on blood samples collected at baseline and three months
2. Autonomic cardiovascular neuropathy measured using a physical examination and ECG at baseline and three months
3. Blood pressure variability measured using 24-hour ambulatory blood pressure monitoring (oscillometric method) from baseline to three months
4. Gut microbiota pattern measured using Next generation sequencing (NGS) of stool samples from baseline to three months
5. Urinary albumin/creatinine ratio assessed by immunoturbidimetry from baseline to three months

Completion date
28/02/2026

Eligibility

Key inclusion criteria
1. Diagnosis of type 2 diabetes mellitus
2. Older than 30 years of age
3. Regular follow-up with an Endocrinologist
4. Treatment with oral antidiabetic medication at a stable dose for at least 3 months prior to visit 1
5. GFR ≥ 60 mL/min/1,73m 2 for átients with UACR ≥ 30 and ≤ 300mg/g
6. Patients with diabetic renal disease using antihypertensive drugs with nephroprotective effect at a stable dose for at least 4 weeks prior to visit 1
7. 25(OH)D levels ≤ 40 ng/ml

Participant type(s)
Patient

Healthy volunteers allowed
No

Age group
Adult

Lower age limit
30 years

Sex
All

Key exclusion criteria
1. Diagnosis of type 2 or other diabetes types
2. 25(OH)D levels ≤ 12 ng/ml
3. Previous and concomitant history of metabolic bone diseases, liver disease
4. Patients that made use of vitamin D or calcium within the last 3 months
5. Hypo or hyperthyroidism unregulated
6. Breastfeeding, pregnant or women who intend to
7. Comorbidities that could interfere with patients' life expectations according to the researcher's opinion

Date of first enrolment
01/08/2023

Date of final enrolment
28/02/2025

Locations

Countries of recruitment

Brazil

Study participating centre
University Hospital João de Barros Barreto, Federal University of Pará
R. dos Mundurucus, 4487 - Guamá
Belém
Brazil
66073-000

Sponsor information

Organisation
Federal University of Para

ROR
https://ror.org/03q9sr818

Funder(s)

Funder type
Other

Funder Name
Investigator initiated and funded

Funder Name
Universidade Federal do Pará

Alternative Name(s)
Federal University of Pará, Universidade Federal do Pará (UFPA), Ufpa_official, UFPA

Funding Body Type
Government organisation

Funding Body Subtype
Universities (academic only)

Location
Brazil

Results and Publications

Individual participant data (IPD) sharing plan
The datasets generated during and/or analysed during the current study are/will be available upon request from João Soares Felicio (felicio.bel@terra.com.br).

IPD sharing plan summary
Available on request