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CBmeter study: protocol for assessing the predictive value of peripheral chemoreceptor overactivation for metabolic diseases

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ABSTRACT

Introduction Early screening of metabolic diseases is crucial since continued undiagnostic places an ever-increasing burden on healthcare systems. Recent studies suggest a link between overactivated carotid bodies (CB) and the genesis of type 2 diabetes mellitus. The non-invasive assessment of CB activity by measuring ventilatory, cardiac and metabolic responses to challenge tests may have predictive value for metabolic diseases; however, there are no commercially available devices that assess CB activity. The findings of the CBmeter study will clarify the role of the CBs in the genesis of—metabolic diseases and guide the development of new therapeutic approaches for early intervention in metabolic disturbances. Results may also contribute to patient classification and stratification for future CB modulatory interventions.

Methods This is a non-randomised, multicentric, controlled clinical study. Forty participants (20 control and 20 diabetics) will be recruited from secondary and primary healthcare settings. The primary objective is to establish a new model of early diagnosis of metabolic diseases based on the respiratory and metabolic responses to transient 100% oxygen administration and ingestion of a standardised mixed meal.

Analysis Raw data acquired with the CBmeter will be endorsed against gold standard techniques for heart rate, respiratory rate, oxygen saturation and interstitial glucose quantification and analysed a multivariate analysis software developed specifically for the CBmeter study (CBview). Data will be analysed using clustering analysis and artificial intelligence methods based on unsupervised learning algorithms, to establish the predictive value of diabetes diagnosis.

Ethics The study was approved by the Ethics Committee of the Leiria Hospital Centre. Patients will be asked for written informed consent and data will be coded to ensure the anonymity of data.

Dissemination Results will be disseminated through publication in peer-reviewed journals and relevant medical and health conferences.

Strengths and limitations of this study

- Assessment of ventilatory, cardiac and metabolic integrated responses to transient hyperoxia and a standardised mixed meal will depict carotid bodies (CBs) activity in a non-invasive manner.
- Preclinical studies have already demonstrated that CB activity correlates with the pathophysiological evolution and severity of metabolic but there is still no evidence that this observation translates into human physiology.
- Physiological data acquisition by the CBmeter prototype needs to be established against gold-standard techniques.
- The test performed with the CBmeter is considered time-consuming, taking about 2 hours to complete, however, other methodologies to assess metabolism, like the oral glucose tolerance test have similar, or longer, completion times.
- The participants in the diabetic group may be recruited at different stages of the disease, and it may be necessary to group patients into clusters for data analysis.

INTRODUCTION

Metabolic diseases are frequently asymptomatic, evolving unnoticed for several years and often diagnosed due to the manifestation of acute or chronic complications.[1] The research for new methods of early diagnosis is essential to prevent the development of complications and to improve the management of metabolic diseases.

The first preclinical study demonstrating the correlation between type 2 diabetes mellitus (T2DM) and overactivity of the carotid bodies (CBs) in animal models was published in 2013.[2 3] Since then, the concern on the role of the CB in the pathophysiology of metabolic diseases is increasingly rising. The CBs are peripheral chemoreceptors, located bilaterally at the bifurcation of the common carotid arteries, that detect and respond to changes in the arterial blood concentrations of oxygen (O_2), carbon dioxide (CO_2) and pH levels.[4] The classical stimulus for CB is

hypoxia, which activates the sympathetic nervous system, allowing normalisation of blood gases through hyperventilation, tachycardia and increases in blood pressure.[4]

The classical tool to look at CB function is assessing the respiratory chemoreflex by measuring the latency of ventilatory responses after an acute hyperoxic challenge.[4] The hyperoxic test or Dejour's test—as it is still known—is a dynamic respiratory functional test in which baseline respiration is recorded while volunteers breathe 21% O_2, for 45 s, followed by 100% O_2 for 20 s. Respiratory variables are analysed for 20 s during 21% O_2 and during the last 15 s of hyperoxia, to assess the sensitivity of the CB.[5] The decrement in ventilatory responses, as a consequence of O_2-breathing, represents the component of the ventilatory drive attributed to the peripheral chemoreceptors. Described since 1962, the Dejour's test never reached clinical relevance probably due to the scarce knowledge on the pathophysiological involvement of CB function in clinically relevant diseases. The paradigm shifted and nowadays it is increasingly evident that the CB coordinates not only acid-base and blood gases homoeostasis but also metabolic regulation, which increased scientific interest in the peripheral chemoreceptors. Supporting these findings, exposure to hyperoxia has been demonstrated to impact glycaemic homoeostasis by suppressing the increase in counterregulatory hormones that occurs under conditions of insulin-induced hypoglycaemia in humans.[6 7] Also, chronic intermittent hyperoxia has been associated with a reduction in fasting and postprandial glucose levels in diabetes patients.[8] Recently, it was demonstrated that CB chemosensitivity is significantly increased in patients with pre-diabetes and that peripheral chemosensitivity correlates with fasting insulin and insulin resistance, representing a novel non-invasive functional biomarker to forecast early metabolic disease.[3]

Thus, both preclinical and clinical data sustain the hypothesis that CB are metabolic regulators and that the diagnosis of CB function may have predictive value for the development of metabolic diseases in humans. Currently, there is no medical device commercially available that associates the diagnosis of CB function with metabolic variables and it is not common clinical practice to associate these organs with endocrine disruption.

The peripheral chemoreceptors are involved in the genesis and progression of pandemic noncommunicable diseases, like T2DM,[9] heart failure[10] and resistant hypertension,[11] thus the evaluation of CB function, as a means to evaluate disease risk, to diagnose subclinical pathologies or even to stratify patients for CB modulatory interventions, becomes a societal and scientific hot topic. There is the need for a device that measures CB activity, designed to perform synchronous longitudinal recordings of cardiorespiratory variables and metabolic parameters in response to challenges that acutely disrupt homoeostasis. The proposed device, the CBmeter, measures the latency of ventilatory and cardiovascular responses not only to hyperoxia but also to a meal challenge test, to assess both the classical respiratory chemoreflex but also the innovative 'metabolic chemoreflex'. Herein, we describe the protocol used in the CBmeter study intended to establish the CBmeter as an integrated system designed for early diagnosis of dysmetabolism.

METHODS AND ANALYSIS

Study design

The CBmeter study is a prospective multicentric, non-randomised controlled interventional study that will be conducted in Portugal for 18 months. Volunteers diagnosed with pre-diabetes or T2DM will be recruited for the experimental group and volunteers without pre-diabetes or T2D for the control group. The study will be conducted in accordance with the Declaration of Helsinki, being previously approved by the Ethics Committee of the Leiria Hospital Centre (Protocol number PI.NC.EC.2018.01). Figure 1 shows the flow diagram of the project's protocol.

Participants

Participants will be recruited in two healthcare centres in Portugal. Volunteers with pre-diabetes or T2DM will be recruited at centre 1 and non-prediabetic/non-diabetic volunteers will be recruited at centre 2. Eligible participants will be adults aged between 25 and 75 years who do not have a diagnosis of respiratory disease. Volunteers who accept to participate will sign the informed consent form. The eligibility of volunteers will be evaluated at visit 0 (V0), after the assessment of inclusion and exclusion criteria. The detailed participant inclusion and exclusion criteria are listed in boxes 1 and 2.

Data collection

Biomarkers

Peripheral venipuncture will be performed to collect 10 mL of venous blood from the antecubital fossa of the left or right upper limb for EDTA coated tubes to measure fasting glucose and insulin concentrations. Insulin concentrations will be measured by ELISA with a DIAsource INS-ELISA Kit (DIAsource ImmunoAssays, Belgium). Fasting glucose will be assessed using the hexokinase method with a commercial kit RX Series GL 3881 (Randox Laboratories, UK).

Dietary intake assessment

Participants' dietary intake will be assessed using two semiquantitative and qualitative interviewer-administered questionnaires. The 24-hour dietary recall is a retrospective questionnaire used to assess participant's food intake within the 24 hours preceding the day of the clinical study. This questionnaire allows the calculation of caloric intake and the distribution of macronutrients and micronutrients on the last day.[12] The Food Frequency Questionnaire recalls the participants' food intake in the last year.[13]

Body composition assessment

Bodyweight (kg), height (cm) and waist circumference (cm) will be measured according to the guidelines for anthropometric procedures in adults from the Portuguese

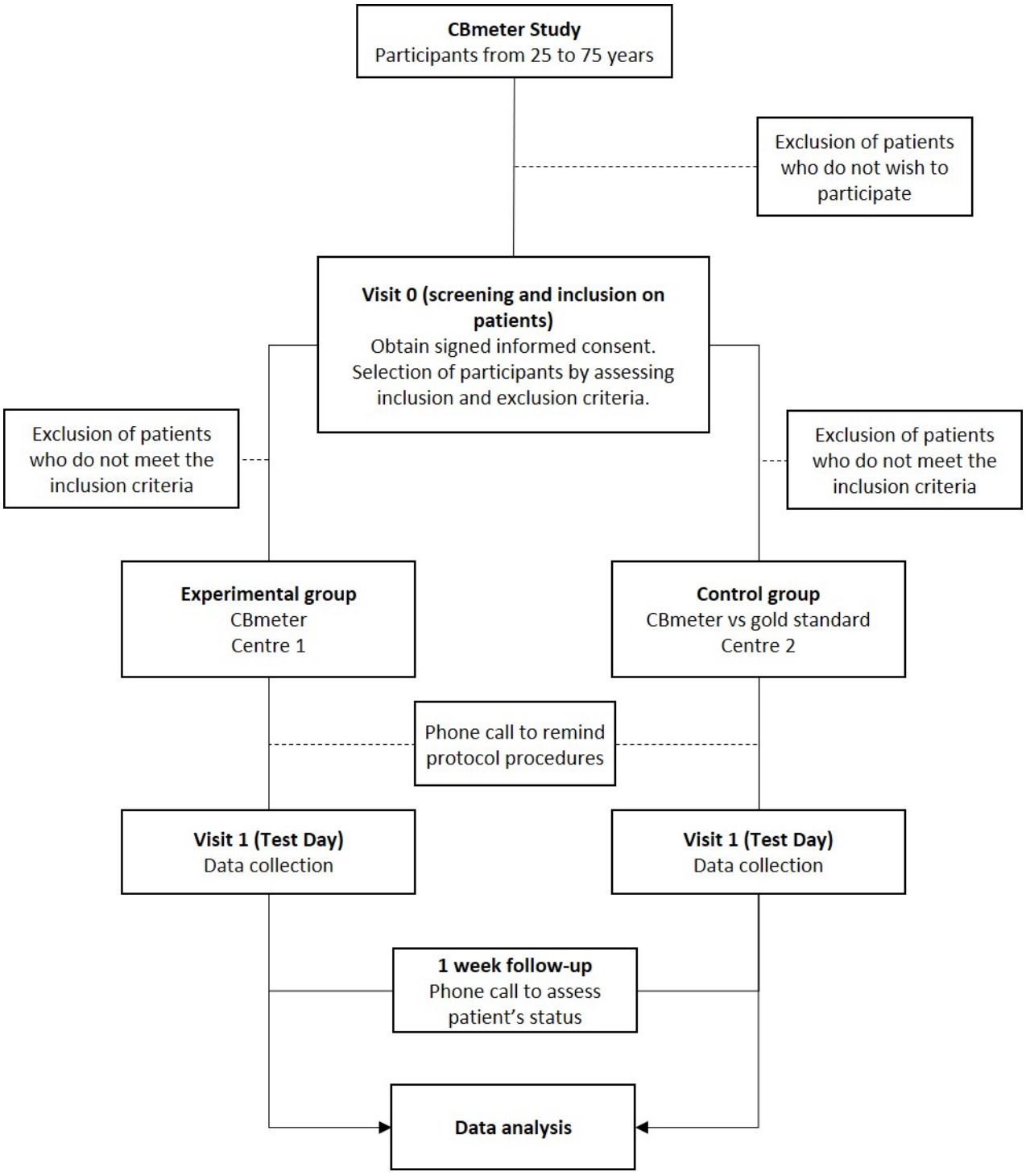

Figure 1 Project flow chart. Participants will be recruited in two healthcare centres: volunteers with pre-diabetes or T2DM will be recruited at centre 1 and healthy volunteers will be recruited at centre 2. Eligible participants will be adults aged between 25 years and 75 years who do not have a diagnosis of respiratory disease. Volunteers who accept to participate in the study will sign the informed consent form. The eligibility of volunteers will be assessed at visit 0 (V0) after verification of inclusion and exclusion criteria. Visit 1 will be scheduled for testing. Data will be collected and analysed and patients will be reminded by a phone call on the day before the test. One week after the test, patients will receive a new phone call to assess their health status. T2DM, type 2 diabetes mellitus.

Directorate General of Health and the ISAK (International Society for the Advancement of Kinanthropometry) International Standards for Anthropometric Assessment guidelines.[14 15] Body fat (%), total body water (%), muscle mass (kg), bone mineral mass (kg), basal metabolic rate (kcal) and visceral fat will be measured using a segmental body composition monitor in one of the centres. Bodyweight (kg), body fat (%) and total body water (%) will be

Box 1 Inclusion and exclusion criteria for the control group of the CBmeter study

Control group

Inclusion criteria

- Adults aged ≥25 and ≤75 years.
- Occasional plasma glucose ≤140 mg/dL with no prior history of diabetes.
- Body mass index 22–29.9 kg/m^2.
- Peripheral oxygen saturation (SpO2) >95%.
- Ability to communicate reliably with the investigators and meet study requirements.

Exclusion criteria

- Prior diagnosis of pre-diabetes or diabetes.
- Previously diagnosed psychiatric illness.
- Previously diagnosed kidney or hepatobiliary disease.
- A chronic respiratory disease.
- Moderate-to-severe sleep apnoea previously diagnosed.
- On antihypertensive medication with beta blockers and thiazide diuretics.
- Food allergy, food intolerance or atopy previously diagnosed.
- Coeliac disease previously diagnosed.
- Vegan.

measured in the second study centre. The difference in the body composition parameters analysed is due to the availability of equipment in the two centres. Body mass index (BMI, kg/m^2) will be calculated and categorised according to age-specific and sex-specific BMI cut-offs for adults.[16] Table 1 resumes the procedures that will be used for anthropometric measurements.

Box 2 Inclusion and exclusion criteria for the experimental group of the CBmeter study

Experimental group

Inclusion criteria

- Adults aged ≥25 and ≤75 years.
- Pre-diabetes or type 2 diabetes mellitus, according to the criteria of the American Diabetes Association[1].
- No pharmacotherapy or metformin monotherapy or Dipeptidyl Peptidase-4 (DPP-IV) inhibitors (gliptins) or in combination (metformin+gliptin).
- Body mass index 22–39.9 kg/m^2.
- Peripheral oxygen saturation (SpO2)>95%.
- Ability to communicate reliably with the investigators and meet study requirements.

Exclusion criteria

- Antidiabetic drugs other than metformin or DPP-IV inhibitors (gliptins) or in combination (metformin+gliptin) administered up to 3 months before the study.
- Previously diagnosed hepatobiliary or renal disease.
- A chronic respiratory disease.
- Moderate-to-severe sleep apnoea previously diagnosed.
- On antihypertensive medication with beta-blockers, calcium channel blockers and thiazide diuretics.
- Food allergy, food intolerance or atopy previously diagnosed.
- Coeliac disease previously diagnosed.
- Vegan.

CBmeter

Heart rate (HR), respiratory rate (RR), peripheral oxygen saturation (SpO_2) and circulating glucose values will be assessed using the CBmeter device. In the control group, the parameters will also be measured using a gold standard methodology to allow comparison with the CBmeter acquisition system and assess its precision and accuracy.

HR will be computed from the ECG signal using a single-lead differential bipolar sensor (biosignalsplux SENSPRO-ECG1-30-30-30). This sensor consists of three surface electrodes placed on the chest, to allow less intrusive recordings compared with the classical ECG setting, and an electronic circuit to measure the electric activity of the heart. RR will be measured using respiratory inductance plethysmography (RIP) sensor (biosignalsplux SENSADV-RIP1), and peripheral O_2 saturation will be assessed through a peripheral capillary O_2 saturation sensor (biosignalsplux SENSPRO-SpO2). A flash glucose monitoring system (FreeStyle Libre Abbott) will be used to measure interstitial tissue glucose levels. The data will be collected using a real-time physiological signal acquisition and processing system at an acquisition rate of 500 Hz, except for interstitial glucose (iGlu) which will be acquired every minute. Baseline values will be collected for 10 min. Participants will be submitted to transient hyperoxia consisting of two deep inhalations of 100% O_2 using high-flow O_2 masks followed by 10 min of data collection with the CBmeter prototype. This manoeuvre will be repeated in triplicate to assess the reproducibility and precision of the CBmeter system. Afterwards, volunteers will be offered a standard mixed meal to be ingested in 10 min and data acquisition will be reinitiated for 60 min. The control group will be evaluated with the CBmeter prototype and simultaneously with a gold-standard ergometer to confirm the accuracy of the CBmeter data acquisition. Data acquisition protocol is represented in figure 2.

Validation of the CBmeter against gold-standard ergometry

To validate the values acquired with the CBmeter sensors, the volunteers will be tested with the CBmeter during the experimental procedure and simultaneously, assessed with a gold-standard test for the acquired electrophysical signals: electrocardiography, spirometry and oximetry, by means of a Cosmed Quark Cardiopulmonary Exercise Testing (CPET) ergometry system (figure 3). Cosmed Quark CPET is a stationary and modular system designed for Pulmonary Function Testing that allows accurate and repeatable tests over time will be used for validating the CBmeter prototype in the present protocol. The CPET system allows simultaneous analysis of the reactions of the cardiac, pulmonary, vascular and metabolic systems to different challenge tests, namely physical activity, with the assessment of O_2 consumption, CO_2 production, minute

Table 1 Procedures for assessment of the anthropometric measurements

Parameter (unit)	Procedure, accuracy and equipment
Height (cm)	► Measured with the head in horizontal Frankfort plane ► Sequentially perform two measurements ► Recorded with a precision of 0.1 cm ► Portable Stadiometer SECA 217
Bodyweight (kg)	► Measured in light indoor clothes and without shoes ► Feet in a parallel position, upright, motionless, head upright, gaze straight ahead and arms extended along the body with palms facing inward ► Sequentially perform two measurements ► Recorded with a precision of 0.1 cm ► Segmental body composition monitor TANITA BC-601 (centre 1) and body composition monitor TANITA SC-240 (centre 2)
Waist circumference	► Measured at the point midway between the iliac crest and the costal margin (lower rib) on the anterior axillary line in a resting expiratory position ► Sequentially perform two measurements ► Recorded with a precision of 0.1 cm ► Stretch-resistance tape SECA 201

ventilation, tidal volume, respiratory exchange ratio, 12-lead ECG, blood pressure and oximetry.[17 18]

Ventilation parameters and respiratory gases assessment requires the use of a Hans Rudolph Oro-Nasal mask. This mask will be chosen according to the size of the face and placed tight fitting to prevent leaks to ensure the highest level of quality in the test.

The ECG will be used to measure HR from the RR-interval. The placement of the chest electrodes will be in the standard positions, namely, the electrodes in the arms will be located in the infraclavicular fossae, and the remained electrodes will be positioned in right and left leg in the anterior axillary line, halfway between the costal margin and iliac crest. In total, there will be 10 electrodes from which all 12 leads can be obtained. This system will be used to compare and validate the signals acquired with the CBmeter prototype with all measurements acquired and integrated in real time. An agreement analysis to determine the accuracy and precision of the CBmeter will be performed. Accuracy will be calculated by assessing how the observed values acquired with the CBmeter agree with the true values evaluated by ergometry) using the Wilcoxon signed-rank test. Precision will be assessed by measuring the extent to which repeated observations with the CBmeter conform during triplicate experiments of responses to hyperoxia using the British standards reproducibility coefficient, to establish a measure of the within-observer agreement.

Challenge tests to assess CB function

To assess CB activity in a non-invasive manner, homoeostasis must be challenged in order to evoke responses by the peripheral chemoreceptors. CB responsiveness will be measured after instigating a change to physiological variables known to be perceived by the peripheral chemoreceptors. The respiratory chemoreflex will be determined using the classical manoeuvre of measuring cardiorespiratory responses after transient inhalation of

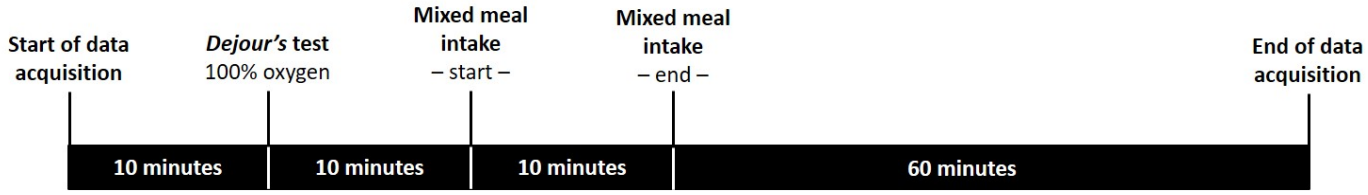

Figure 2 Data acquisition protocol. Heart rate, respiratory rate (RR), peripheral oxygen saturation (SpO2) and circulating glucose values will be assessed using the CBmeter prototype. Heart rate will be computed from the ECG signal using a single-lead differential bipolar sensor (biosignalsplux SENSPRO-ECG1-30-30-30). RR will be measured using respiratory inductance plethysmography sensor (biosignalsplux SENSADV-RIP1); peripheral oxygen saturation will be assessed through a peripheral capillary oxygen saturation (SpO2) sensor (biosignalsplux SENSPRO-SpO2). A flash glucose monitoring system (FreeStyle Libre Abbott) will be used to measure interstitial tissue glucose levels. The data will be collected at an acquisition rate of 500 Hz, except for interstitial glucose which will be acquired every minute. Baseline values will be collected for 10 min. Afterwards, the patients will be submitted to transient hyperoxia consisting of two deep inhalations of 100% oxygen using high-flow oxygen masks followed by 10 min of data collection using the CBmeter prototype. Afterwards, the patients will be offered a standard Mixed-meal to be ingested in a 10 min period. Following, data acquisition will be reinitiated for 60 min. The control group of patients will be assessed simultaneously with the CBmeter prototype and with a gold-standard ergometer to validate the accuracy of the CBmeter data acquisition.

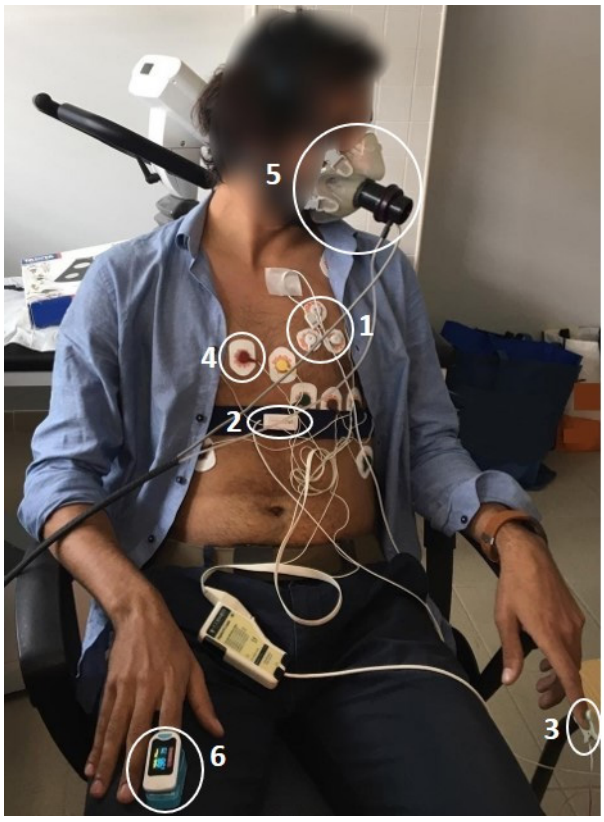

Figure 3 Representative figure of placement of the CBmeter electrodes together with the ergometry electrodes for heart rate, respiratory rate and peripheral oxygen saturation assessment. 1—CBmeter heart rate electrodes; 2—CBmeter respiratory rate thoracic band; 3—CBmeter peripheral oxygen saturation (SpO2) sensor; 4—Ergometer ECG electrodes; 5—Ergometer Hans Rudolph Oro-nasal mask; 6—SpO2 gold-standard assessment by a fingertip pulse oximeter.

100% medicinal O_2. Participants will perform two successive inhalations of medicinal O_2 that will be administered through a high concentration mask. The manoeuvre will be repeated three times to assess reproducibility of the recordings. RR, SpO_2 and HR will be recorded continuously, allowing to assess the respiratory chemoreflex.

The innovative metabolic chemoreflex will be assessed by measuring the ventilatory, cardiac and metabolic responses after administration of a standard mixed meal, specifically developed for the CBmeter protocol. The participants will ingest the standard mixed meal to trigger the metabolic response while the changes in interstitial glucose, HR, RR and SpO_2 are recorded continuously. The standard mixed meal has 400 kcal and consists of 65% of carbohydrates, 23% of protein and 12% of lipids, which corresponds to 20% of the average energy intake for an adult.

Software: CBview

CBview is a software tool developed to visualise, process and analyse the physiological signals acquired by the CBmeter system.[19] It is an intuitive graphical user interface that analyses the recordings of physiological responses acquired with the CBmeter, providing quantitative metrics to assess metabolic dysfunction. CBview was developed using the MatLab computing platform. The software has an initial configuration tab and four other different tabs each one assigned to a different physiological signal: ECG, RIP, SpO2 and iGlu. After processing the ECG signal (peak detection and time interval computation), CBview constructs an HR variability (HRV) tachogram that depicts the RR-interval evolution along time. Spectral analysis of the HRV tachogram is used to evaluate the influence of each subsystem constituting the autonomous nervous system. The RR variability derived from the RIP output data is another valuable result. The CBview software also provides a graph representing the blood O_2 level variation along time based on the SpO_2 photoplethysmography signal.[19] Test positivity cut-offs for diabetes and non-diabetes features will be determined with the exploratory results obtained herein. Considering the nature of the data, analysis methodologies based on classical statistical measures will not meet the sensitivity required in nonlinear systems (eg, the human body trying to obtain homoeostasis). To overcome this 'CBView' executes temporal studies and also analysis in the frequency domain, which will be complemented in the following prespecified studies with additional functionalities regarding time-frequency domain processing techniques and chaos theory.[19]

Calculation of sample size and study potency

Using available data from patients with T2DM and hypertension who underwent a pilot study for RR assessment and response to Dejour's test at NOVA Medical School[3] and assuming a criterion of the significance of 5%; statistical power of 80% and a coefficient of variation of 5%—Power and Precision Software V.4, the minimum number of patients required for the experimental group will be 20 participants.

Data sharing and protection

The data collection and database are fully compliant with the General Data Protection Regulation (Regulation (European Union) 2016/679 of the European Parliament and the Council of 27 April 2016) and the Portuguese Clinical Investigation Law no. 21/2014, of 16 April. The study is conducted following the principles of the International Conference Harmonisation-Good Clinical Practice. Study results will be disseminated in the form of conference abstracts and peer-reviewed publications.

Patient and public involvement

Patients and the public were not involved in the design, or conduct, or reporting, or dissemination plans of our research project. The results of this work will be disseminated to the participants and the public via conferences, publications and presentations.

Ethics and dissemination

The Ethics Committee of Leiria Hospital Centre approved the CBmeter study, which is conducted according to the principles of the Declaration of Helsinki, and the

Portuguese Clinical Research Law no. 21/2014, of 16 April. Changes in protocol and amendments will be approved by the Ethics Committee and registered before implementation. This study is covered by liability insurance. Informed consent will be obtained from participants before any data are collected. Participants have the right to withdraw from the study at any time, without the need to provide a reason if they wish not to do so. Participants will be assured that if they wish to withdraw from the study, their medical care will not be affected. Besides, the investigator can discontinue a participant from the study at any time if the investigator considers it necessary for any reason. The reason for that withdrawal must be recorded in the case report form, including safety and clinical reasons, study compliance or if they withdraw their consent. Except if the participant indicates otherwise, any data collected up to the point of withdrawing consent will be included in the final analysis. The collected data will be coded to guarantee the confidentiality and anonymity of the participants, according to the General Data Protection Regulation. Participants will only have access to their data on request to the principal investigator.

The results will be disseminated through publication in peer-reviewed journals and presentations at international scientific meetings.

DISCUSSION

The decisive point for managing diabetes is early diagnosis, since the longer a person lives with undiagnosed and untreated diabetes, the worse their health outcomes are expected to be. In patients with obesity, pre-diabetes or T2DM, symptoms are often absent or less marked when compared with those of type 1 diabetes mellitus and, as a result, the disease might go undiagnosed for years, until complications have already started to arise. These negative effects, including a higher risk of diabetes-related complications, increase healthcare systems use and related costs.[20 21] The methods currently used to diagnose metabolic diseases can only detect advanced stages of these diseases. Several tools that assess the risk of having undiagnosed or future diabetes have been developed and adapted for use in diverse populations.[22] However, the majority of these are based on clinical parameters, clinical history and anthropometric measures that may not be able to detect subclinical manifestations of disease development. Early diagnosis of metabolic diseases makes it possible to treat and adopt preventive measures that avoid complications. This early detection can be based on a quantitative test that will underline the recommendations of lifestyle changes by healthcare professionals. From a motivational point of view, it could be easier for patients to adopt healthy eating habits and lifestyles when there is an altered biomarker that indicates subclinical disease, particularly in a condition that remains asymptomatic until advanced stages of their pathocrony. Thus, early detection of subtle disease patterns, like changes in HR and RR in response to blood gases variations or a glucose load may be of utmost importance for such behaviours to be implemented as early as possible, increasing effective health gains and decreasing health expenditures. Cunha-Guimaraes *et al*[3] showed that CB are overactive in prediabetic subjects and that peripheral chemosensitivity correlates with fasting insulin and insulin resistance, which can represent a new non-invasive method to forecast early metabolic disease. However, currently, there is no medical device available that associates the diagnosis of CB function with metabolic function. Thus, the development of the CBmeter device and its integrated software, the CBview, is a ground-breaking approach. This device was designed to be minimally invasive reducing the risks related to its use as a diagnostic method in a clinical context. It is not expected that there are any adverse effects arising from participation in this study.

The development of the standardised mixed meal as an alternative to the oral glucose solution used to perform the oral glucose tolerance test (OGTT) can also be considered an innovative aspect. The OGTT solution does not represent the common dietary pattern and can cause side effects such as vomiting, diarrhoea and bloating. Besides, the information is limited to capillary glucose and insulin and can only be used to diagnose diabetes and pre-diabetes.[23] The standard mixed meal allows a more gradual elevation in iGlu than that observed after the OGTT, making it more suitable for testing potential diabetes and pre-diabetes, as well as for reducing the risk of hypoglycaemia and associated complications.

The limitation of this innovative method is related to the duration of the test. The acquisition of physiological signals takes about 2 hours, which is similar to the time it takes to perform OGTT, which is currently considered the gold standard method for diagnosing pre-diabetes or diabetes. In the future, we intend to improve and overcome this limitation with this test in an outpatient setting.

Ultimately, we believe this data-driven methodology may represent a valuable tool to understand the evolution of respiratory, cardiac, gasometric and metabolic parameters in early phases of metabolic diseases grounding the basis for faster and better metabolic diseases diagnosis.

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Contributors MPG and RF-P originated the idea for the study. MPG and RF-P were involved in the funding acquisition and managing of the project. MPG, RF-P, SF, LC and AV contributed to the design of the study, development of the research protocol and statistical methods. MPG and ML drafted the manuscript and revised it critically for intellectual content. All authors read and approved the manuscript submitted.

Competing interests None declared.

Patient and public involvement Patients and/or the public were not involved in the design, or conduct, or reporting, or dissemination plans of this research.

Patient consent for publication Not required.

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