Carotid body chemosensitivity: early biomarker of dysmetabolism in humans

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Abstract

Objective: The carotid bodies (CBs) are peripheral chemoreceptor organs classically described as being O_2 sensors, which are increasingly emerging as core players in metabolic control. Herein we evaluated CB activity in prediabetes patients and determined its correlation with dysmetabolism clinical features.
Design and methods: Prediabetes patients were recruited at the Cardiology Service, Hospital Santa Marta, Centro Hospitalar Lisboa Central, EPE (CHLC-EPE). The study was approved by CHLC-EPE and NOVA Medical School Ethics Committee. Thirty-three prediabetic and 14 age-matched, non-prediabetic, volunteers had their peripheral chemosensitivity evaluated by the Dejours test. Serum biomarkers of metabolic disease, insulin sensitivity (HOMA-IR), blood pressure, carotid intima-media thickness (cIMT) and glucose tolerance were assessed.
Results: CB chemosensitivity was significantly increased in prediabetic group ($P < 0.01$). Fasting blood, glucose intolerance, fasting insulin and HOMA-IR were significantly higher in prediabetes patients. Insulin resistance correlated both with peripheral chemosensitivity, assessed by the Dejours test ($P < 0.05$) and with abdominal circumference ($P < 0.01$). HbA1c correlated with HOMA-IR ($P < 0.05$) and left cIMT ($P < 0.05$) in prediabetes patients.
Conclusions: We conclude that CB is overactive in prediabetes subjects and that peripheral chemosensitivity correlates with fasting insulin and insulin resistance representing a novel non-invasive functional biomarker to forecast early metabolic disease.

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Introduction

The carotid bodies (CBs) are peripheral chemoreceptors that classically sense changes in arterial blood O_2, CO_2 and pH levels (1). The classical stimulus for the carotid body is hypoxemia, which leads to an increase in CSN activity. Besides its role in the cardiorespiratory control, the CB has been proposed as a metabolic sensor implicated in the control of energy homeostasis (2, 3). There has been huge debate in the literature on the hypothesis that CBs sense glucose and that hyperglycemia may be the trigger for CB overactivation observed in metabolic diseases. However, scientific evidence supports that carotid bodies, although having a fundamental role in glucose homeostasis, do not sense glucose directly except in acute hypoglycemic states where there is urgent need to regulate the counterregulatory response and appropriate increase in ventilation. In metabolic disease states, hyperinsulinemia, rather than hyperglycemia, appears to be the key trigger for CB overactivation (4, 5). Recently, our group described that CB overactivation is involved in the development of insulin resistance and

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glucose intolerance (5, 6) core features of very prevalent diseases like the metabolic syndrome and type 2 diabetes via activation of the sympathetic nervous system (7, 8, 9, 10). We showed that animal models of diet-induced prediabetes develop an increased CB chemosensitivity (5, 7, 9) that results in an increase in SNS activity and in a reduction in insulin sensitivity (4, 7, 8, 9). All these characteristic features of metabolic diseases were prevented by CSN resection disclosing CB's primordial role in the control of peripheral insulin sensitivity (4, 5, 6, 7, 8, 9, 11). In agreement with this increased activation of the CB in dysmetabolic states, the CBs of prediabetic and type 2 diabetic animals are hyperplasic and exhibit a higher percentage of neurosecretory type 1 cells, confirmed by the higher expression of tyrosine hydroxylase (4, 10), a molecular marker for this type of cells. Confirming this preclinical data, the CBs of type 2 diabetic patients that are 20–25% larger than control volunteers (12).

Additionally, the abolishment of CB activity in animals, via chronic resection of CB sensitive nerve, the carotid sinus nerve (CSN) (4, 9, 11) or, alternatively, through its bioelectronic modulation (11) prevented and reversed dysmetabolism in animal models of prediabetes and type 2 diabetes, respectively, by positively impacting glucose uptake and insulin signaling in the liver and in the visceral adipose tissue (8) and by normalizing whole-body sympathetic activity (8, 9). More recently, we showed that the diabetic condition can be discriminated on the basis of CSN and sympathetic neural activities due to a high-frequency shift in both spectra (8), being the sympathetic neural activity shift suppressed upon CSN denervation, confirming the role of CSN in driving sympathetic overactivation in type 2 diabetes. Indeed, augmented peripheral chemosensitivity has been shown to contribute not only to type 2 diabetes, but also to several other sympathetic-mediated diseases, as essential hypertension, hypertension associated with sleep apnea and heart failure, both in animal models (13, 14, 15, 16, 17, 18) and in humans (19, 20, 21, 22, 23).

In agreement with our pre-clinical data demonstrating the involvement of CB dysfunction in the development of peripheral insulin resistance and glucose intolerance, we have shown that hyperbaric oxygen therapy, a therapeutic approach frequently used to promote wound healing to treat diabetic foot, improves glucose homeostasis in type 2 diabetes patients (24), an effect that is probably mediated by CB inhibition, as hyperoxia dramatically reduces peripheral chemoreceptor activity (25).

Thus, the CBs emerge as an innovative therapeutic target for patients with metabolic diseases associated to overactivation of the sympathetic nervous system (26), but evidence is still lacking as to the relation between increased CB firing rate and the subclinical development of dysmetabolism in humans.

The general aim of the present work was to investigate if CB chemosensitivity is altered in prediabetes patients. The hypothesis tested was that CB chemosensitivity is augmented in prediabetes and that CB activity correlates with classical prediabetes disease features.

Methods

Ethical approval

The study was approved by Hospital Santa Marta, Centro Hospitalar Lisboa Central EPE (CHLC-EPE, n°63/2010) and NOVA Medical School Ethics Committee and performed in accordance with the Helsinki Declaration. Written informed consent was obtained from all individuals.

Subjects

Prediabetes patients were recruited at the Cardiology Service, Hospital Santa Marta, CHLC-EPE. Inclusion criteria for prediabetes were those defined by the American Diabetes Association (27). Exclusion criteria were cardiovascular disorders, except hypertension, renal diseases, obesity hypoventilation syndrome, chronic respiratory failure, and psychiatric diseases. Thirty-three prediabetic patients (age = 64.1 ± 11.2 years, HbA1c = 6.0 ± 0.5%, 36.36% male:63.63% female) exclusively under non-pharmacological treatment for glycemic control and 14 non-prediabetic controls (age = 60.6 ± 13.4 years, HbA1c = 5.5 ± 0.3%; 50% male:50% female) were included. The clinical study was conducted in two visits.

Study design and subjects monitoring

The first visit was at CHLC-EPE where sociodemographic and anthropometric data, comorbidities and ongoing medication profile were documented. Weight, height and abdominal circumference using standardized protocols were assessed. Blood pressure was measured, and hypertension defined by a previous diagnosis of hypertension or the presence of systolic blood pressure ≥140 mmHg or diastolic blood pressure ≥90 mmHg (mean of two consecutive measurements). Fasting peripheral blood was collected by venopuncture to assess

Table 1 Demographic, cardiometabolic and ventilatory variables in non-prediabetes individuals (n = 14) and prediabetic (n = 33) patients. Values are presented as mean ± S.D.

Characteristics	Non-prediabetics	Prediabetics	*P* value*
Age, years	60.57 (3.43)	64.06 (11.20)	0.359
BMI	27.21 (7.36)	30.15 (4.56)	0.114
Abd. circumference, cm	88.71 (15.64)	96.67 (12.95)	0.077
Systolic BP, mmHg	136.60 (11.72)	152.90 (20.04)	**0.007**
Diastolic BP, mmHg	83.57 (8.43)	82.48 (12.67)	0.769
Gender (Male:Female), %	(50/50)	(36.36/63.63)	
Insulin, µUI/mL	6.30 (3.72)	10.50 (7.26)	**0.047**
HOMA-IR	1.34 (0.88)	2.55 (2.21)	0.054
HbA1c, , %	5.59 (0.31)	6.04 (0.52)	**0.004**
Cholesterol, mg/dL	198.40 (26.64)	186.60 (37.21)	0.289
HDL-c, mg/dL	57.07 (14.10)	50.03 (15.23)	0.146
LDL-c, mg/dL	132.40 (23.39)	124.10 (29.97)	0.361
TAG, mg/dL	116.10 (52.21)	155.10 (96.28)	0.162
Apo A1, mg/dL	140.10 (36.75)	141.70 (25.19)	0.863
Apo B, mg/dL	102.30 (18.62)	90.90 (22.36)	0.101
CRP, mg/dL	4.43 (9.88)	4.80 (4.76)	0.862
Dopamine, pg/mL	93.65 (55.07)	109.75 (136.66)	0.702
Epinephrine, pg/mL	21.48 (13.93)	33.33 (25.42)	0.132
Norepinephrine, pg/mL	276.60 (149.90)	326.20 (207.80)	0.458
Fasting plasma glucose, mg/dL	67.13 (9.31)	90.85 (20.89)	**0.004**
2-h glycemia, mg/dL	120.40 (12.36)	165.60 (41.09)	**0.005**
RR baseline, bpm	11.86 (2.94)	12.30 (3.30)	0.738
VT, L/kg	6.39 (2.18)	8.00 (2.17)	0.074
Decrease RR, %	4.62 (15.43)	−8.48 (10.62)	0.010
NO, µM	56.45 (25.06)	64.49 (57.35)	0.687
cIMT right, mm	0.80 (0.14)	0.83 (0.18)	0.661
cIMT left, mm	0.82 (0.17)	0.814 (0.17)	0.925

* One-Way ANOVA.

Abd. Circumference, abdominal circumference; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; BMI, body mass index; cIMT, carotid intima-media thickness; CRP, C-reactive protein; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; LDL, low-density lipoprotein; NO, nitric oxide; RR, respiratory rate; TAG, triglyceride; VT, tidal volume.SI conversion factors: To convert Abd. Per. to m, multiply values by 0.01. To convert Insulin to pmol/L, multiply values by 6.9488. To convert cholesterol, HDL-c, LDL-c, TAG, Apo A1, Apo B, fasting glycemia, 2-h glycemia to mmol/L, multiply values by 0.0259. To convert dopamine, epinephrine, norepinephrine to pmol/L, multiply values by 0.2926. To convert CIMT left, CIMT right to m, multiply values by 0.001.

fasting plasma insulin, fasting blood glucose, HbA1c, lipid profile as well as other biological parameters (Table 1). Plasma samples were analyzed in the hospital central laboratory and biological parameters quantified using automatic standard routine enzymatic methods. HDL cholesterol was determined after specific precipitation. LDL cholesterol was determined by Friedewald formula. Catecholamines in plasma, assessed as an indirect measurement of sympathetic nervous system activity, were quantified by high performance liquid chromatography with electrochemical detection. Carotid intima-media thickness (cIMT) was assessed by high-resolution B-mode ultrasonography with a Siemens Sonolite™ system and a 7.5-MHz linear array transducer. In magnified and frozen images, cIMT was measured at the distal common carotid artery (1 cm proximal to the carotid bifurcation) at the far wall. Manual measurements of cIMT were obtained from both, the right and the left side carotids. The lumen/intima leading edge to media/adventitia leading edge method was used (28). cIMT value used for the present analysis was defined as the maximum value between the right and left common carotids.

The second visit was performed at NOVA Medical School to assess glucose tolerance and peripheral chemosensitivity by the double breath Dejours test (29, 30). Glucose tolerance status was determined by means of an oral glucose tolerance test (OGTT), the standard measure for both glucose intolerance and diabetes diagnosis. Briefly, the OGTT consisted in the oral administration of a glucose drink with orange flavor containing 75 g glucose (Top Star 75, Toplabs, Portugal) and in the measurement of blood glucose before and 2 h after ingestion. The OGTT was performed in fasting volunteers on the day the Dejours test was performed. Fasting blood glucose

levels were quantified with a glucometer (Precision Xtra Meter, Abbott Diabetes Care, Portugal) and test strips (Abbott Diabetes Care, Portugal). To perform the Dejours test, ventilation, namely respiratory frequency (RR) and tidal volume (VT) were measured, while subjects breathed room air (21%O_2; normoxia) followed by two breaths of 100%O_2 (hyperoxia) delivered at a 10 L/min flow and by normoxia again, via a mouthpiece connected to a three-way valve in a whole-body plethysmography system (MasterScreen Body, Jaeger, Germany). Hyperoxia applied during a few seconds resulted in a decrease in ventilation that reflects CB chemosensitivity (29, 30) and can be expressed as a percentage of the pre-oxygen breathing respiratory frequency. % decrease in RR and VT was calculated as follows: mean of RR or VT values during the period of time from which 100% O_2 is applied to the time where RR or VT values return to baseline values minus the mean of RR or VT during exposure to air (Fig. 1A). The maneuver was repeated three times in each patient to assess reproducibility of the test.

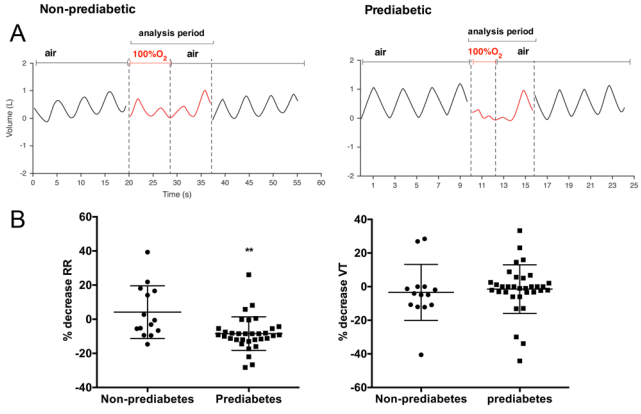

Figure 1

Carotid body chemosensitivity in non-prediabetes and prediabetes subjects. (A) Show typical spirograms for the effect of 100% O_2 (hyperoxia) on the spontaneous ventilation in non-prediabetes (Left panel A) and in prediabetes (Right panel A) individuals; (B) left panel depicts carotid body chemosensitivity evaluated as the % of decrease in respiratory rate produced by two breaths of 100% O_2 (Dejours test). Right panel shows the absence of differences between prediabetes and non-prediabetes patients on % of decrease in tidal volume. Two breaths of hyperoxia were applied to evaluate CB sensitivity. Red line shows the period of time used to calculate mean respiratory rate (RR) and mean tidal volume (VT) to access the effect of hyperoxia on these variables. Values are mean ± S.D. $^{**}P < 0.01$.

Statistical analysis

Statistical analysis was performed using the GraphPad Prism Software, version 6 and PASW 18.0 program (SPSS Inc). *P* values of 0.05 or less were considered to represent significant differences.

Quantitative variables were described as mean and standard deviation. Normality was assessed with Kolmogorov–Smirnov test. Qualitative variables are presented as percentages. ANOVA test or Kruskal–Wallis test was used for between group comparisons of continuous variables (according to distribution characteristics), while chi-square test was used for between group comparisons of categorical variables.

To investigate the variables associated with CB chemosensitivity in prediabetic patients we evaluated Spearman's correlation coefficient. Multivariable analysis using a stepwise multiple linear regression analysis was performed to study the association between the defined independent variables and CB chemosensitivity, as dependent variable (Table 3). Also, a stepwise multiple linear regression analysis was performed considering HOMA and HbA1c, as dependent variables (Table 4).

Results

Demographic, cardiometabolic and ventilatory variables in controls (non-prediabetes patients) and prediabetics are depicted in Table 1 and drug therapy in Table 2. Fasting and 2-h blood glucose following OGTT were 94.3 ± 16.2 mg/dL and 165.6 ± 41.1 mg/dL in prediabetes ($P < 0.001$), and 84.5 ± 9.9 mg/dL and 120.4 ± 12.3 mg/dL in control patients, respectively. Plasma fasting insulin levels and HOMA-IR were 10.5 ± 7.3 µUI/mL ($P < 0.01$) and 2.4 ± 2.2 µUI/mL ($P < 0.05$), in prediabetes volunteers, and 6.3 ± 3.7 µUI/mL and 1.3 ± 0.9 µUI/mL in controls. No significant differences were observed in the lipid profile between non-prediabetes and prediabetes subjects. Total cholesterol, HDL-c, LDL-c and triglycerides levels were, respectively, 186.6 ± 37.2 mg/dL, 50.03 ± 15.2 mg/dL, 124.1 ± 30.0 mg/dL, 155.1 ± 52.2 mg/dL in prediabetics and 198.40 ± 26.6 mg/dL, 57.1 ± 14.1 mg/dL, 132.4 ± 23.4 mg/dL and 116.1 ± 52.21 in non-prediabetics. Figure 1A depicts representative spirograms of basal ventilation (black line, air) and Dejours test (1st 2 breaths of the red line) in a non-prediabetes and in a prediabetes volunteer. The Dejours test has proven to be reproducible in specific populations (31) and we also tested its reproducibility, by performing three replicate tests in each participant. No significant

Table 2 Risk factors and drug therapy in non-prediabetes individuals (n = 14) and prediabetic (n = 33) patients. Variables are presented as n (%).

	Non-prediabetics	Prediabetics	*P* value
Risk factors, *n* (%)			
Smoking	3 (21.43)	5 (14.71)	0.534
Coffee	7 (50.00)	20 (58.82)	0.646
Hypertension	0	8 (23.53)	**0.048**
Dyslipidemia	1 (7.14)	9 (26.47)	0.140
Medication, *n* (%)			
β-blocker	1 (7.14)	12 (35.29)	**0.047**
ARB	3 (21.43)	12 (35.29)	0.357
Statin	5 (35.71)	14 (41.18)	0.732
Calcium-channel blocker	3 (21.43)	7 (20.59)	0.949
Diuretics	4 (28.57)	7 (20.59)	0.560
ACE inhibitor	4 (28.53)	13 (38.24)	0.535
Fibrates	0	3 (8.82)	0.260
Anti-platelet agents	0	3 (8.82)	0.260

*$P < 0.05$, one-way ANOVA.
ARB, angiotensin receptor blocker; ACE. angiotensin-converting enzyme.

variation among the replicates was observed (data not shown). In our experimental setting, we observed that the prediabetic subject presents both a higher tidal volume (VT, P=0.074) and a higher respiratory rate (RR, P=0.738) (Fig. 1A and Table 1) in comparison with the non-prediabetic. As expected, 2 breaths of 100%O_2 decreased ventilation (Fig. 1). A statistically significant increase in CB chemosensitivity was detected in prediabetic patients since the % of decrease in RR, produced by 2 breaths of 100%O_2, was higher in this group than in the non-prediabetes group as depicted in the representative spirograms of Fig. 1 (% decrease RR prediabetes=-8.48±10.62 vs %decrease RR controls=4.62±15.43, P<0.01, Fig. 1A and B). No differences were seen between the prediabetic and non-prediabetic group on the % of decrease in tidal volume produced by 2 breaths of 100% O_2 (% decrease VT controls=−3.43±16.66%; Fig. 1B right panel).

Right and left cIMT were 0.83±0.2 mm and 0.81±0.2 mm, respectively, in prediabetes patients and 0.80±0.1 mm and 0.82±0.2 mm, in the control group. Spearman analysis found significant correlations between CB chemosensitivity and both, insulin levels (r=−0.58, P<0.01), and HOMA-IR (r=−0.46, P<0.05), but no correlation among other variables tested (Supplementary Table 1, see section on supplementary materials given at the end of this article).

Lastly, using a multivariable analysis, by the stepwise method, we found that in prediabetes subjects, the dependent variable Dejours test only correlated with the independent variable, plasma fasting insulin levels (P=0.042) and, therefore, with HOMA-IR (Table 3). Additionally, the dependent variables HOMA-IR and HbA1c correlated with the independent variables: abdominal circumference (P=0.006) and Dejours test (P=0.026) and with HOMA-IR (P=0.018) and left cIMT (P=0.043), respectively (Table 4).

Discussion

Herein we tested the proof of concept that augmented CB chemosensitivity is a clinical feature in prediabetes. The main novel finding is that CB chemosensivity correlates with fasting plasma insulin levels and with HOMA-IR index in prediabetes patients, classical markers of insulin resistance. This study carries a significant innovative potential since it shows that CB overactivity in humans may represent a non-invasive and accurate functional biomarker of hyperinsulinemia and insulin resistance.

Although being a pilot study, the results are statistically robust and show that prediabetes patients exhibit increased CB chemosensitivity and that disrupted CB activity patterns are linked to circulating insulin and to insulin resistance. Herein, CB chemosensitivity was evaluated by 2 breaths of 100%O_2, the double-breath Dejours test (29, 30), as with this test is possible to obtain a change in the oxygen drive almost free of secondary factors since they are secondary in time (30, 32). Indeed, if 100%O_2 is breathed during prolonged exposures (several minutes) ventilation does not change or even can increase (32). The increased CB chemosensitivity observed in prediabetes patients was mainly seen in RR but not in tidal volume (Fig. 1B). This increased CB chemosensitivity, clearly manifested in the % of reduction in RR was also

Table 3 Multivariable analysis between the dependent variable carotid body chemosensitivity (% decrease in RR) and the independent variables using the stepwise method.

Dependent variable / Independent variable	**CB chemosensitivity** (% decrease RR)	
	Unstandardized coefficients B	*P* value
Univariable Analysis – Linear Regression – Enter Method		
Age	−0.174	0.449
BMI	0.004	0.994
Abd. Circumference	−0.112	0.581
Systolic BP	−0.056	0.670
Diastolic BP	−0.173	0.425
Gender	8.904	**0.097**
Insulin	−0.848	**0.017**
HOMA	–	–
HbA1c	−1.214	0.830
Cholesterol T	0.028	0.711
HDL-C	−0.158	0.368
LDL-C	0.026	0.795
TAG	−0.003	0.941
ApoA1	−0.114	0.278
ApoB	0.036	0.799
CRP	0.567	0.378
Dopamine	-0.010	0.702
Epinephrine	0.207	**0.117**
Norepinephrine	−0.018	0.329
Fasting plasma glucose	–	–
2h Glycemia	0.043	0.487
RR Baseline	–	–
Tidal volume	–	–
Dejour Test (% decrease RR)	–	–
Conc NO	0.049	0.296
CIMT right	−28.966	**0.104**
CIMT left	−6.871	0.652
Multivariable Analysis – Linear Regression – Stepwise Method		
Insulin	−0.841 (−1.648: −0.035)*	0.042

Variables to be tested in the multivariable analysis are chosen using the variables with a value of $P < 0.25$ in the univariable analysis.
*Figures in parentheses indicate 95% CI for B.
Abd. Cir, abdominal circumference; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; BMI, body mass index; CIMT, carotid intima-media thickness; CRP, C-reactive protein; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; LDL, low-density lipoprotein; MV, minute volume; NO, nitric oxide; RR, respiratory rate; TAG, triglyceride.

previously seen in chronic heart failure patients (20, 33) as well as in animal studies in mice (34, 35).

The increased CB chemosensitivity observed in prediabetes patients in the present manuscript confirms our pre-clinical work in where we described an increased CB activity in prediabetes and type 2 diabetes rodent models, measured as increased neurotransmitters released from CB type cells (4, 6) by increased carotid sinus nerve basal activity (5, 7, 8) and by increased spontaneous minute ventilation (4) and also supports the clinical imaging study performed by Cramer *et al*. describing that the CBs of type 2 diabetes patients are 20–25% larger than control volunteers, indicating enlarged over functioning organs in the diabetic population (12). Additionally, it supports our hypothesis that insulin is a trigger for CB activation (3, 4, 5, 6, 36) and the recent results by Vidal *et al*. (37) in where they show that insulin promotes hepatic glycogenolysis by acting on the CB. In agreement also with our findings, that CB chemosensitivity correlates with fasting plasma insulin levels, is the work by Barbosa *et al*. (38) where they show in humans that elevated plasma insulin increases minute ventilation independently of changes in glucose. More recently, the same authors examined the contribution of the CB chemoreceptors to the insulin-mediated increases in muscle sympathetic nerve activity (MSNA) in healthy humans and found that the attenuation of CB activity by low-dose dopamine or by hyperoxia, did not altered insulin-mediated increase MSNA burst frequency, suggesting that CB withdrawal in young, healthy men and women did not attenuate the sympathoexcitatory response to hyperinsulinemia (39). If at a first sight these results seem conflicting with our hypothesis that increased CB chemosensitivity promotes glucose homeostasis disruption due to the overactivation of the sympathetic nerve system, they are not, as we previously concluded that reductions in CB chemoreceptor activity positively affects insulin signaling pathways in visceral adipose tissue and liver, but not in skeletal muscle (9). Indeed, the study of Limberg *et al*. (39) corroborate our own since we also did not observe changes in glucose uptake in muscle after CSN ablations (9).

We observed no correlation between CB activity and cIMT or fasting blood glucose. This contrasts to what was previously suggested in disease paradigms of sleep apnea (40, 41), where increased cIMT has been proposed to be the trigger to CB overactivation, through hypoperfusion of the organs; and also in type 2 diabetes, where increased fasting blood glucose levels have been proposed as a stimuli to the CBs (42). Thus, in our experimental setting, overactivation of the CBs observed in prediabetes patients was not attributable to ischemic hypoxia of the carotid chemoreceptors, nor to hyperglycemia since our population was normoglycemic. The significant correlation disclosed between CB chemosensitivity and fasting insulin levels strongly suggests that insulin and not hypoxia or hyperglycemia is the main trigger for CB overactivation in prediabetes, as we previously reported in animal models of metabolic diseases. This is also in agreement with our rodent work in where we showed that hyperglycemia did not modify CSN activity (5).

Table 4 Multivariable analysis between the dependent variables HOMA and HbA1c and the independent variables using the stepwise method.

Dependent variable / Independent variable	HOMA		HbA1c	
	Unstandardized coefficients B	*P*-value	Unstandardized coefficients B	*P* value
Univariable Analysis – Linear Regression – Enter Method				
Age	−0.004	0.907	0.134	0.498
BMI	0.218	**0.009**	0.363	**0.057**
Abd. Circumference	0.090	**0.004**	0.318	**0.100**
Systolic BP	0.030	**0.112**	0.401	**0.038**
Diastolic BP	-0.006	0.832	0.197	0.325
Gender	-1.290	**0.130**	-0.205	0.294
Insulin	–	–	–	–
HOMA	–	–	0.679	**<0.001**
HbA1c	–	–	–	–
Cholesterol T	−0.004	0.695	0.083	0.676
HDL-C	−0.042	**0.112**	−0.290	**0.134**
LDL-C	−0.002	0.877	0.109	0.581
TAG	0.006	**0.173**	0.367	**0.055**
ApoA1	−0.017	0.326	−0.289	**0.152**
ApoB	0.016	0.400	0.345	**0.084**
CRP	0.061	0.477	0.116	0.557
Dopamine	−0.004	0.436	−0.107	0.694
Epinephrine	−0.028	**0.206**	−0.105	0.670
Norepinephrine	0.002	0.387	0.380	**0.109**
Fasting plasma Glucose	0.025	**0.230**	–	–
2h Glycemia	0.007	0.441	–	–
RR Baseline	–	–	0.302	**0.183**
Tidal volume	0.155	0.447	0.224	0.329
DejoursTest (% decrease RR)	−0.068	**0.051**	−0.051	0.830
Conc NO	0.002	0.730	0.210	0.336
CIMT right	1.580	0.474	0.491	**0.077**
CIMT left	1.824	0.436	0.373	**0.050**
Multivariable Analysis – Linear Regression – Stepwise Method[†]				
Abdominal Per.	0.121 (0.042, 0.199)*	0.006	–	–
CB Chemosensitivity (%)	−0.082 (−0.153, −0.012)*	0.026	–	–
HOMA	–	–	0.098 (0.020, 0.175)*	0.018
CIMT left	–	–	1.176 (0.044, 2.308)*	0.043

Variables to be tested in the multivariable analysis are chosen using the variables with a value of $P < 0.25$ in the univariable analysis.
*Figures in parentheses indicate 95% CI for B; [†]All variables that were not statistically significant in each multivariable model were excluded.
Abd. Circumference, abdominal circumference; ApoA1, apolipoprotein A1; ApoB, apolipoprotein B; BMI, body mass index; CIMT, carotid intima-media thickness; CRP, C-reactive protein; HbA1c, glycated hemoglobin; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; LDL, low-density lipoprotein; MV, minute volume; NO, nitric oxide; RR, respiratory rate; TAG, triglyceride.

We observed no significant differences amongst non-prediabetes individuals and prediabetic patients in the risk factors assessed, except for hypertension which was not present in the control group volunteers; nor drug therapy, except for beta-blockers, which were more prevalent in the prediabetic group than in control group. CB overactivation has also been recently associated with the development of essential and resistant hypertension (13, 43). Thus, we tested the hypothesis that different responses to hyperoxia observed were not due to significantly different blood pressure. Surprisingly, although a significant difference in systolic blood pressure among groups was observed, no correlation was found between CB activity and this variable: the multivariable analysis between the dependent variable CB chemosensitivity (% decrease in RR) and the independent variable systolic blood pressure did not show a significant correlation among the variables ($P=0.670$) indicating that, despite the differences found between the study population and control group, blood pressure is not related to CB overactivation.

This is not in agreement with the previous findings by Narkiewicz *et al.*, describing that pathological afferent signaling emanating from CB drives sympathetically mediated elevations in blood pressure in resistant hypertension patients, a different study population that may justify the disparities with our study (44). Also, deactivation of CB chemoreceptors by hyperoxia decreased blood pressure in hypertensive patients (33)

and CB unilateral ablation was associated to no change in blood pressure. However, eight patients showed significant reductions in ambulatory blood pressure overlapping with decreases in sympathetic activity (44); nevertheless, conclusions are difficult to drawn since metabolic parameters were not assessed in this pool of patients. Based on our findings, we believe that CB activity does not correlate directly with blood pressure in metabolic diseases patients, which suggests that aberrant discharges by the CBs lead to different disease phenotypes, according to the type of fibers activated in the carotid sinus nerve or the type of stimuli perpetrated. Further studies are required to disclose the selective modulation of specific nerve fibers in the CBs by distinct triggers.

In conclusion, we found that CB augmented chemosensitivity is a clinical feature of prediabetes that correlates with circulating plasma fasting insulin levels and with insulin sensitivity a core feature of dysmetabolism. This discovery conveys groundbreaking potential for early screening and diagnosis of metabolic diseases, based on non-invasive evaluation of CB activity, allowing identification of individuals that, lacking clinical signs of metabolic disease, are silently developing dysmetabolism promoted by CB overactivation and are putative candidates to therapeutic CB-specific modulation.

Supplementary materials

This is linked to the online version of the paper at https://doi.org/10.1530/EJE-19-0976.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this study.

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Author contribution statement

Prof. Silvia Conde and Miguel Mota Carmo are the guarantors of the study. They had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Author contributions are as follows: Study concept and design: Conde, Mota-Carmo, Guarino; Acquisition of data: Timóteo, Mota-Carmo, Selas, Conde, Caires, Sacramento, Ribeiro, Santiago; Analysis and interpretation of data: Conde, Guarino, Cunha-Guimarães, Mota-Carmo; Statistical analysis: Conde, Cunha-Guimarães, Timóteo; Drafting of the manuscript: Conde, Guarino; Critical revision of the manuscript for important intellectual content: all authors have reviewed and contributed to the intellectual content of the manuscript; Obtained funding: Conde; Administrative, technical, or material support: Conde, Selas, Caires; Study supervision: Conde, Mota-Carmo. Miguel Mota-Carmo and Silvia V Conde: both authors have contributed equally as senior authors to this manuscript.

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