<https://helda.helsinki.fi>

Hyperoxia improves autonomic function in individuals with long-duration type 1 diabetes and macroalbuminuria

Laursen, J.C.

2020-09

Laursen , J C , Hansen , C S , Bordino , M , Vistisen , D , Zobel , E H , Winther , S A , Groop , P-H , Frimodt-Møller , M , Bernardi , L & Rossing , P 2020 , ' Hyperoxia improves autonomic function in individuals with long-duration type 1 diabetes and macroalbuminuria ' , Diabetic Medicine , vol. 37 , no. 9 , pp. 1561-1568 . <https://doi.org/10.1111/dme.14315>

<http://hdl.handle.net/10138/329527>

<https://doi.org/10.1111/dme.14315>

unspecified

acceptedVersion

Downloaded from Helda, University of Helsinki institutional repository.

This is an electronic reprint of the original article.

This reprint may differ from the original in pagination and typographic detail.

Please cite the original version.

DR JENS CHRISTIAN LAURSEN (Orcid ID : 0000-0002-5036-8159)

DR CHRISTIAN STEVNS HANSEN (Orcid ID : 0000-0002-5782-3476)

Article type : Research Article

Diabetic Medicine

Article type: Research Article

Article number: DME-2019-00778

Copy editor: Maria Hale

Proofs to: jens.christian.laursen.01@regionh.dk

Copyright: Diabetes UK

Author running head: J. C. Laursen *et al.*

Short title running head: Autonomic function in long-duration type 1 diabetes and macroalbuminuria

Hyperoxia improves autonomic function in individuals with long-duration type 1 diabetes and macroalbuminuria

J. C. Laursen^{1,2}, C. S. Hansen¹, M. Bordino^{3,4,5}, D. Vistisen¹, E. H. Zobel¹, S. A. Winther¹, P-H. Groop^{3,4,5,6}, M. Frimodt-Møller¹, L. Bernardi^{3,4,5} and P. Rossing^{1,2}

¹Steno Diabetes Center Copenhagen, Gentofte and ²University of Copenhagen, Copenhagen, Denmark, ³Folkhälsan Institute of Genetics, Folkhälsan Research Center, Biomedicum Helsinki, ⁴Abdominal Center Nephrology, University of Helsinki and Helsinki University Hospital, ⁵Research Program for Clinical and Molecular Medicine, Faculty of Medicine, University of Helsinki, Helsinki, Finland, and ⁶Department of Diabetes, Central Clinical School, Monash University, Melbourne, Victoria, Australia

Correspondence to: Jens Christian Laursen. E-mail: jens.christian.laursen.01@regionh.dk

What's new?

- Cardiovascular autonomic dysfunction is a risk factor for mortality in type 1 diabetes.
 - Hyperoxaemia can acutely improve autonomic function in individuals with short-duration type 1 diabetes.
 - We explored the potential for improving autonomic function in long-duration type 1 diabetes with macroalbuminuria, and found that autonomic function could be improved.
 - Autonomic dysfunction in type 1 diabetes might be a dynamic and reversible condition.
- Hypoxia might represent a target of intervention for attenuating progression of autonomic dysfunction in type 1 diabetes.

Abstract

Aim Acute oxygen inhalation and slow deep breathing improve measures of autonomic function transiently in individuals with short-duration type 1 diabetes. Our aims were to examine these interventions and changes in autonomic function in individuals with long-duration type 1 diabetes and to explore interactions with the presence of macroalbuminuria or existing cardiovascular autonomic neuropathy.

Methods Individuals with type 1 diabetes ($n = 54$) were exposed to acute oxygen inhalation, slow deep breathing and a combination of both (hereafter 'the combination'). Primary outcomes were change in baroreflex sensitivity and heart rate variability. Associations between changes in outcomes were evaluated using mixed effects models.

Results Mean age $\pm$ SD was 60 ± 10 years and diabetes duration was 38 ± 14 years. Changes are presented as per cent difference from baseline with 95% confidence intervals. Acute oxygen inhalation, slow deep breathing and the combination increased baroreflex sensitivity by 21 (10, 34)%,

32 (13, 53)% and 30 (10, 54)%, respectively. Acute oxygen inhalation trended towards increasing heart rate variability 8 (−1, 17)% ($P = 0.056$), and slow deep breathing and the combination increased heart rate variability by 33 (18, 49)% and 44 (27, 64)% respectively. Macroalbuminuria or cardiovascular autonomic neuropathy did not modify results.

Conclusion Autonomic function is improved transiently in individuals with long-duration type 1 diabetes and normoalbuminuria or macroalbuminuria by acute oxygen inhalation and slow deep breathing. There is a risk of survival bias. Autonomic dysfunction might be a reversible condition, and hypoxia might represent a target of intervention.

<H1>Introduction

Diabetic neuropathy is a serious and prevalent complication in individuals with type 1 diabetes and type 2 diabetes. A prevalent form is cardiovascular autonomic neuropathy (CAN) [1]. CAN is a risk factor for mortality [2–4] as well as a risk marker for the development of micro- and macrovascular complications such as diabetic nephropathy and cardiovascular disease [5–8].

The progression of CAN can be attenuated by intensive glycaemic control in individuals with type 1 diabetes, as shown by the Diabetes Control and Complications Trial (DCCT) [9], and by multifactorial therapy targeting glycaemia, blood pressure (BP), lipids and lifestyle in individuals with type 2 diabetes, as shown by the Steno 2 study [10]. It has also been suggested that CAN is reversible in the early stages [11]. Early CAN may therefore be considered a functional cardiovascular autonomic dysfunction and not necessarily a result of irreversible structural nerve damage. Recent studies demonstrated that slow deep breathing (six deep breaths per min) and acute oxygen inhalation (normobaric 100% oxygen nasally, 6 l/min) transiently improved measures of cardiovascular autonomic function such as baroreflex sensitivity and heart rate variability [12–14]. These studies

were carried out in short-duration uncomplicated type 1 diabetes, and it is not known whether the same changes can be found in long-duration type 1 diabetes and in those with macroalbuminuria.

The aim of the study was to examine the interventions acute oxygen inhalation and slow deep breathing on changes in baroreflex sensitivity and heart rate variability in individuals with long-duration type 1 diabetes, and to explore whether the presence of macroalbuminuria or existing CAN interacted with these changes. The main hypothesis for the study was that CAN, manifested as reduced baroreflex sensitivity, is a dynamic and reversible condition also in the late stages of type 1 diabetes. Previous studies have shown improvements in cardiovascular autonomic function after interventions causing hyperoxaemia, the hypothesis being that relieving tissue hypoxia plays an important role in the mechanisms of the effects [12–14]. We addressed this by examining the secondary outcomes, blood oxygen saturation and tissue oxygen saturation in the arm.

<H1>Methods

<H2>Study population

Study participants were recruited at Steno Diabetes Center Copenhagen as part of a cross-sectional study investigating myocardial flow reserve and coronary artery calcium score in normo- or macroalbuminuric individuals with type 1 diabetes [15]. Normoalbuminuria was defined as urinary albumin excretion < 30 mg/24 h or < 30 mg/g creatinine in two of three consecutive measurements, and macroalbuminuria was defined as > 300 mg/24 h or > 300 mg/g creatinine in two of three consecutive measurements within 1 year. No participants had microalbuminuria (30–300 mg/g creatinine).

All participants in the original study (60) were invited to participate in the current study; 54 accepted. Of these, 29 were from the original normoalbuminuria group and 25 were from the original macroalbuminuria group (24 of these were on renin angiotensin–aldosterone system blocking treatment). Urine albumin was not measured again for this study.

Participants were > 18 years of age with a diagnosis of type 1 diabetes (WHO criteria). Main exclusion criteria were the presence of: non-diabetic kidney disease; end-stage renal disease (eGFR < 15 ml min⁻¹ 1.73 m⁻²), dialysis or kidney transplantation; change in renin angiotensin–aldosterone system blocking treatment during the past month.

The study complies with the Declaration of Helsinki, and the research protocol was approved by the Ethical Committee of the Capital Region of Copenhagen, review number 56623. All participants gave written informed consent.

<H2>Clinical measurements

HbA_{1c} was measured by high-performance liquid chromatography. Plasma creatinine was measured by an enzymatic method (Hitachi 912, Roche Diagnostics, Mannheim, Germany). The CKD-EPI equation was used to calculate eGFR. Urinary albumin-to-creatinine ratio was measured by an enzymatic immunoassay in three consecutive morning urine samples. Three measurements of brachial BP were obtained after 5 min in sitting position with an oscillometric BP monitor (um201, A&D Medical, Tokyo, Japan). Height and weight were measured, and BMI calculated as weight/height (kg/m²). A detailed medical history including treatment and previous cardiovascular disease was obtained from all participants and cross-referenced with electronic patient records. Current smoking was defined as one or more cigarettes/cigars/pipes a day.

<H2>Experimental protocol

Participants were fasting and abstinent from medication and tobacco from midnight the day before the visit and refrained from strenuous exercise 24 h prior to the examination. Testing was performed in a quiet, temperature-controlled room at 20–26 °C, between 08:00 and 13:00. The experimental protocol included simultaneous measurement of continuous electrocardiogram (ECG) (bipolar precordial leads), digital continuous BP (Finapres 2300 digital plethysmograph; Ohmeda, Louisville, CO, USA) from the first finger of the left hand held at heart level, respiration rate (inductive

respiratory belts positioned around the chest and abdomen), pulse oximetry (Cosmo; Novamatrix, Wallingford, CT, USA) and a near-infrared spectroscopy skin sensor on the forearm. All signals were acquired simultaneously on a laptop, using an analogue-to-digital converter with a 12-bit precision at a sampling rate of 400Hz (Software: DataloggerControl-205-BT) [16]. Measurements were performed with the participant in the supine position.

Measurements were done in test cycles, each including three recordings. A 300-s measurement with spontaneous breathing, a 120-s measurement with paced breathing at a standardized respiration rate (15 breaths/min) and a 120-s measurement of slow deep breathing (6 breaths/min). Respiration rate was paced guided by a metronome. The test cycle was repeated while administering normobaric 100% oxygen nasally (6 l/min).

<H2>Interventions

To account for different respiration rates during spontaneous breathing between individuals, standardized paced breathing (at a normal rate of 15 breaths/min in room air) was considered the baseline in the primary analyses. The three interventions were: (1) acute oxygen inhalation (100% oxygen nasally, respiration rate 15 breaths/min); (2) slow deep breathing (room air, respiration rate 6 breaths/min); and (3) a combination of the first two (100% oxygen nasally, respiration rate 6 breaths/min, hereafter ‘the combination’). Secondary analyses were performed with spontaneous breathing as baseline.

<H2>Baroreflex sensitivity and heart rate variability

Baroreflex sensitivity is defined as the extent to which the heart rate changes when BP changes, and is considered a sensitive measurement of cardiovascular autonomic function [17]. Baroreflex sensitivity was determined from spontaneous fluctuations in the ECG-derived time elapsed between two successive R-waves of the QRS signal (RR interval) and systolic BP during the different parts of the test cycle [13,14]. Heart rate variability was evaluated by calculating the standard deviation of all

RR intervals, also known as the standard deviation of the normal to normal interval (SDNN). It was obtained from the time series of the continuous ECG measurements.

<H2>Cardiovascular autonomic neuropathy

Three standard cardiovascular autonomic reflex tests (CARTs) were used to diagnose CAN: changes in heart rate during a lying-to-standing test, deep breathing test and Valsalva manoeuvre. Participants were considered to have 'no CAN' when zero or one abnormal CART were detected and 'CAN' if two or three abnormal CARTs were detected [7]. Age-dependent cut-off levels for the CARTs defined by Cardone [18] and recommended and translated into English by Spallone *et al.* [19] were used. Owing to poor data quality or poor compliance, some participants did not have usable data from at least two CARTs. Because of this, 11 participants were not eligible for evaluation of the CAN diagnosis due to only one or no usable CARTs. CARTs were obtained with the Vagus™ device (Medicus Engineering, Aarhus, Denmark).

<H2>Statistical analysis

Participant characteristics are presented as n (%), mean $\pm$ SD, or if skewed distributions, as median [IQR; quartile 1, quartile 3]. For clinical characteristics, chi-squared test and Fisher's exact test were used for categorical data and t -tests were used for quantitative data.

Primary outcomes were per cent change in baroreflex sensitivity and SDNN after interventions. Secondary outcomes were per cent change in blood oxygen saturation and arm oxygen saturation after interventions. All outcomes were log-transformed with the natural logarithm before analysis to fulfil the requirement of normally distributed model residuals in all models. Associations of changes in outcomes after interventions were evaluated and compared by a mixed-effects model with a patient-specific random intercept, with change in outcome-variables as outcome and the three interventions as exposures. We tested for an interaction between the first two interventions on the

outcomes. This corresponds to testing whether the effect of the third intervention (a combination of the first two) was different from the sum of the effects of the first two interventions.

A modifying effect of albuminuria on the interventions was tested with two levels of confounder adjustment by including an interaction term between the intervention and albuminuria status. First, with adjustment for the effect of the three interventions, baseline values of outcome variables and albuminuria-status (model 1), and with further adjustment for age, diabetes duration, sex, smoking, CAN status and multiple medications (anti-hypertensive treatment, beta-blocking treatment and/or renin angiotensin–aldosterone system blocking treatment) (model 2). A modifying effect of CAN and smoking was tested using the same approach as for albuminuria. Geometric means of the outcomes were calculated at baseline and at follow-up (after interventions). From the mixed-effects models, we estimated the change (per cent difference) from baseline and *P*-values are reported for test of significance of the difference.

Statistical significance was inferred as a two-tailed *P*-value < 0.05. All analyses were performed in SAS 9.4 (SAS Institute, Cary, NC, USA).

<H1>Results

<H2>Participant characteristics

Fifty-four individuals with type 1 diabetes were enrolled. Mean $\pm$ SD age was 60 ± 10 years and mean diabetes duration was 38 ± 14 years. Twenty-five participants (46%) had macroalbuminuria, 12 (22%) had CAN and 7 (13%) were current smokers. Median [IQR] baroreflex sensitivity was 4.8 [2.8, 7.7] ms/mmHg and median SDNN was 9.5 [5.8, 15.9] ms. When participants were split into the normoalbuminuria and macroalbuminuria groups, the macroalbuminuria group had a higher prevalence of elevated coronary artery calcium score, lower baroreflex sensitivity and lower SDNN (Table 1).

<H2>Baroreflex sensitivity and heart rate variability

Acute oxygen inhalation, slow deep breathing and the combination increased baroreflex sensitivity by 21 (10, 34)%, 32 (13, 53)% and 30 (10, 54)%, respectively (Table 2 and Fig. 1a). There was no significant difference in changes between acute oxygen inhalation alone and slow deep breathing alone ($P = 0.507$). There was an interaction between the two ($P < 0.001$), showing no additional change of adding slow deep breathing to acute oxygen inhalation ($P = 0.335$) or vice versa ($P = 0.837$) (Table 2). There was no modifying effect of macroalbuminuria ($P \geq 0.05$), CAN ($P \geq 0.05$) or smoking ($P \geq 0.05$).

Acute oxygen inhalation showed a trend towards increasing SDNN 8 (-1, 17)% ($P = 0.056$). Slow deep breathing and the combination increased SDNN 33 (18, 49)% and 44 (27, 64)%, respectively (Table 2 and Fig. 1b). The change with slow deep breathing was significantly greater than the effect of acute oxygen inhalation alone ($P < 0.001$). There was an interaction between the change with acute oxygen inhalation and slow deep breathing on SDNN ($P < 0.001$), showing a greater change with the combination than with acute oxygen inhalation alone ($P < 0.001$), and a trend towards a difference from slow deep breathing alone ($P = 0.075$) (Table 2). There was no modification of the response of interventions on SDNN of macroalbuminuria ($P \geq 0.05$), CAN ($P \geq 0.05$) or smoking ($P \geq 0.05$).

As a sensitivity analysis, analyses on primary outcomes were repeated after removing smokers ($n = 7$) and the results did not change.

Changes induced by the interventions with spontaneous breathing as the baseline were also modelled.

Important differences were that baroreflex sensitivity was not improved significantly by any of the interventions, although there was a trend towards improvement after acute oxygen inhalation of 20 (-1, 44)% ($P = 0.057$). SDNN was improved by slow deep breathing and the combination, but not by acute oxygen inhalation (Table S1).

<H2>Oxygen saturation levels

Acute oxygen inhalation and the combination increased blood oxygen saturation 1.0 (0.5, 1.4)% and 1.0 (0.7, 1.6)%, respectively, but slow deep breathing did not (Table 2 and Fig. 1c). There was an interaction between the change with acute oxygen inhalation and slow deep breathing ($P < 0.001$). The change with acute oxygen inhalation was greater than that from slow deep breathing ($P < 0.001$) and the change with the combination was greater than that with slow deep breathing alone ($P < 0.001$) (Table 2). There was no interaction of the response of interventions on blood oxygen saturation of macroalbuminuria ($P \geq 0.05$), CAN ($P \geq 0.05$) or smoking ($P \geq 0.05$).

Acute oxygen inhalation and the combination increased arm oxygen saturation 1.0 (0.4, 1.6)% and 0.7 (0.1, 1.2)%, respectively, but slow deep breathing did not (Table 2 and Fig. 1d). There was an interaction between the changes with acute oxygen inhalation and slow deep breathing. The change with acute oxygen inhalation was greater than that with slow deep breathing ($P < 0.001$) and the change with the combination was greater than that with slow deep breathing alone ($P < 0.001$) (Table 2). There was no interaction of the response of interventions on arm oxygen saturation of macroalbuminuria ($P \geq 0.05$), CAN ($P \geq 0.05$) or smoking ($P \geq 0.05$).

Changes induced by the interventions on oxygen saturation levels with spontaneous breathing as baseline were also modelled. Blood oxygen saturation and arm oxygen saturation were both improved by all three interventions (Table S1).

<H1>Discussion

This study had several important findings. In a population of individuals with long-duration type 1 diabetes and normoalbuminuria or macroalbuminuria, acute oxygen inhalation and slow deep breathing improved baroreflex sensitivity and heart rate variability. There was no evidence of an additive change in baroreflex sensitivity by combining the interventions. The observed changes after acute oxygen inhalation seemed to be driven by hyperoxia, whereas the changes after slow deep

breathing seemed to be driven by something else. Finally, there was no effect-modification of albuminuria or existing autonomic dysfunction.

In a previous study using the same method, baroreflex sensitivity in healthy participants was > 18 ms/mmHg [16]. Based on this, the participants in this study clearly had a low baroreflex sensitivity. The even lower baroreflex sensitivity in those with macroalbuminuria compared with normoalbuminuria is not surprising and is in line with findings observed in microalbuminuric individuals with type 1 diabetes [20]. Reduced baroreflex sensitivity in type 1 diabetes is likely to be caused by a range of different mechanisms, some of which may be irreversible and some of which may be reversible. One presumably irreversible factor causing reduced baroreflex sensitivity is calcification of the baroreceptors [21]. The macroalbuminuric group had a higher coronary artery calcium score, supporting this theory. Reversible factors could be hypoxia in the blood or tissue. This is addressed below.

The observation that reduced baroreflex sensitivity and heart rate variability can be improved in individuals with long-duration type 1 diabetes supports the idea that there might be functional component in the pathophysiology of CAN. This has been demonstrated in short-duration type 1 diabetes and the authors proposed a mechanism with relieved tissue hypoxia, in part causing the effects [13]. In the present study, acute oxygen inhalation increased oxygen saturation in the tissue of the arm and in the blood, while also improving autonomic function. This supports the hypothesis that relieving hypoxia contributes to improving autonomic function. Of note, arm tissue oxygenation does not necessarily represent the tissue of interest, i.e. tissue connected with cardiovascular autonomic function and the baroreflex. Measuring oxygenation in the paraventricular nucleus of hypothalamus, the nucleus of the solitary tract, the parasympathetic output nerves, and so forth may be possible theoretically, but is difficult. Measuring oxygen saturation directly in peripheral nerves has been done previously [22] and could serve as a better proxy for general oxygenation levels in nervous tissue in

future studies. Slow deep breathing also improved autonomic function, but with no change in oxygen saturation in the blood or tissue of the arm. In other words, slowing the breathing rate improved autonomic function, independently of the measured oxygenation levels. We found no additive change in baroreflex sensitivity induced by combining the two interventions, suggesting that they work through different mechanisms, as already proposed [16]. On the other hand, for heart rate variability, we found a trend towards an additive change when adding acute oxygen inhalation to slow deep breathing, indicating that the mechanisms could be associated.

The observed changes in baroreflex sensitivity induced by the interventions with spontaneous breathing as a baseline were different from those found in previous studies [13,16]. In the present study, there was a trend towards an improving change induced by acute oxygen inhalation alone, but the effect of slow deep breathing was lost completely. The effect of acute oxygen inhalation on SDNN was lost, whereas the change induced by slow deep breathing on SDNN was reproduced. These differences could be due to high variability in the baseline respiration rate during spontaneous breathing. As stated above, autonomic function is highly dependent on respiration rate. To account for this, and to standardize the participants' baseline values, we considered paced breathing with a respiration rate of 15 breaths/min to be the baseline in the primary analysis.

There was no effect modification of the observed changes by macroalbuminuria. We are aware of the risk of over-fitting when testing for the interaction in model 2. However, the results were similar in model 1 and model 2, supporting the result of no interaction. This is in accordance with previous findings in type 2 diabetes [12]. Keeping in mind that participants with macroalbuminuria had a lower baseline baroreflex sensitivity, this also means that autonomic function in participants with macroalbuminuria was improved, but not normalized. Our interpretation is that there is potential for restoration even when macroalbuminuria is present, but the baseline value might be too low for normalization. This could be due to already advanced changes in the target organs. Another

possibility is that the initial response to hyperoxia is not sufficient for complete recovery due to a lower baseline value and that long-term exposure could have a normalizing effect.

<H2>Strengths and limitations

Limitations of this study are the small sample size, recruitment from a single centre (reducing generalizability) and lack of an age-matched healthy control group. Another limitation is that study participants had either normoalbuminuria or macroalbuminuria, thus representing the extremes of a long-duration type 1 diabetes population in terms of microvascular dysfunction. For comparison between groups, it is a limitation that the two groups are different in several ways, for example in diabetes duration. It should also be noted that participants with macroalbuminuria are survivors, and that individuals with macroalbuminuria and severe autonomic neuropathy who might have had a particularly non-reversible type of autonomic neuropathy may have died before reaching the age of the population in this study. In the analysis of effect-modification by CAN, it is a limitation that the definition of CAN is based on a small sample, as defined by Cardone [18]. Finally, *P*-values have not been adjusted for multiple testing. It is, however, a strength of this study that measurements were performed under standardized conditions including fasting, abstinence from alcohol, smoking and strenuous physical activity, and that the observer, room and equipment were the same for all participants. Equipment, software and methods used for assessment of autonomic dysfunction parameters were adapted from previous studies [12–14], and instruction and training of investigators were done in collaboration with the authors of those studies.

<H1>Conclusion

Autonomic function can be transiently improved in individuals with long-duration type 1 diabetes and normoalbuminuria or macroalbuminuria by acute oxygen inhalation and slow deep breathing. For baroreflex sensitivity, changes induced by interventions were of similar magnitude, but were not

additive. For heart rate variability, maximal change was seen with the combination. Autonomic dysfunction might be a reversible condition. Hypoxia might represent a target for intervention.

Funding sources

Internal funding was provided by Steno Diabetes Center Copenhagen. The authors acknowledge the support from the Novo Nordisk Foundation grant number NNFOC0013659: 'PROTON: Personalizing Treatment of Diabetic Nephropathy'.

Competing interests

P-H.G. reports receiving lecture honoraria from Astellas, Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Elo Water, Genzyme, Medscape, MSD, Mundipharma, Novartis, Novo Nordisk, PeerVoice and Sanofi, and being an advisory board member of AbbVie, Astellas, Astra Zeneca, Boehringer Ingelheim, Eli Lilly, Janssen, Medscape, MSD, Mundipharma, Novartis, Novo Nordisk and Sanofi.

P.R. reports having received research grants from Astra Zeneca and Novo Nordisk, has given lectures for Astra Zeneca, BMS and Boehringer Ingelheim, and has served as a consultant for AbbVie, Astra Zeneca, Bayer, Eli Lilly, Boehringer Ingelheim, Astellas, Gilead, Sanofi Aventis and Novo Nordisk, all fees given to Steno Diabetes Center, and has equity interest in Novo Nordisk. No other conflicts of interest relevant to this article were reported. D.V. reports having stock equity in Novo Nordisk.

Acknowledgements

The authors thank all participants and acknowledge the work of laboratory technicians at Steno Diabetes Center Copenhagen, Denmark.

Author contributions

J.C.L. drafted the manuscript. J.C.L., C.S.H., M.B., E.H.Z. and S.A.W., M.F.M., P-H.G. L.B. and P.R. analysed and interpreted the research. J.C.L., C.S.H., M.B., P-H.G., M.F.M., L.B. and P.R. conceived and designed the research. J.C.L., D.V. and C.S.H. performed the statistical analysis. J.C.L., M.B., E.H.Z and S.A.W. did measurements. C.S.H., P-H.G., M.F.M., L.B. and P.R. supervised the study. P.R. obtained the funding. All authors contributed to the interpretation of the results, reviewed and edited the manuscript and approved the final version of the manuscript. J.C.L. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

References

- 1 Pop-Busui R, Boulton AJ, Feldman EL, Bril V, Freeman R, Malik RA *et al.* Diabetic neuropathy: a Position Statement by the American Diabetes Association. *Diabetes Care* 2017; **40**: 136–154.
- 2 Maser RE, Mitchell BD, Vinik AI, Freeman R. The association between cardiovascular autonomic neuropathy and mortality in individuals with diabetes: a meta-analysis. *Diabetes Care* 2003; **26**: 1895–1901.
- 3 Pop-Busui R, Evans GW, Gerstein HC, Fonseca V, Fleg JL, Hoogwerf BJ *et al.* Effects of cardiac autonomic dysfunction on mortality risk in the Action to Control Cardiovascular Risk in Diabetes (ACCORD) trial. *Diabetes Care* 2010; **33**: 1578–1584.
- 4 Soedamah-Muthu SS, Chaturvedi N, Witte DR, Stevens LK, Porta M, Fuller JH *et al.* Relationship between risk factors and mortality in type 1 diabetic patients in Europe: the EURODIAB Prospective Complications Study (PCS). *Diabetes Care* 2008; **31**: 1360–1366.

- 5 Wheelock KM, Jaiswal M, Martin CL, Fufaa GD, Weil EJ, Lemley KV *et al.* Cardiovascular autonomic neuropathy associates with nephropathy lesions in American Indians with type 2 diabetes. *J Diabetes Complications* 2016; **30**: 873–879.
- 6 Orlov S, Cherney DZI, Pop-Busui R, Lovblom LE, Ficociello LH, Smiles AM *et al.* Cardiac autonomic neuropathy and early progressive renal decline in patients with nonmacroalbuminuric type 1 diabetes. *Clin J Am Soc Nephrol* 2015; **10**: 1136–1144.
- 7 Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R *et al.* Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. *Diabetes Metab Res Rev* 2011; **27**: 639–653.
- 8 Astrup AS, Tarnow L, Rossing P, Hansen BV, Hilsted J, Parving HH. Cardiac autonomic neuropathy predicts cardiovascular morbidity and mortality in type 1 diabetic patients with diabetic nephropathy. *Diabetes Care* 2006; **29**: 334–339.
- 9 The effect of intensive diabetes therapy on the development and progression of neuropathy. The Diabetes Control and Complications Trial Research Group. *Ann Intern Med* 1995; **122**: 561–568.
- 10 Gaede P, Vedel P, Larsen N, Jensen GV, Parving HH, Pedersen O. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med* 2003; **348**: 383–393.
- 11 Burger AJ, Weinrauch LA, D'Elia JA, Aronson D. Effect of glycemic control on heart rate variability in type I diabetic patients with cardiac autonomic neuropathy. *Am J Cardiol* 1999; **84**: 687–691.
- 12 Esposito P, Mereu R, De Barbieri G, Rampino T, Di Toro A, Groop PH *et al.* Trained breathing-induced oxygenation acutely reverses cardiovascular autonomic dysfunction in patients with type 2 diabetes and renal disease. *Acta Diabetol* 2016; **53**: 217–226.

- 13 Bernardi L, Rosengard-Barlund M, Sandelin A, Makinen VP, Forsblom C, Groop PH. Short-term oxygen administration restores blunted baroreflex sensitivity in patients with type 1 diabetes. *Diabetologia* 2011; **54**: 2164–2173.
- 14 Rosengard-Barlund M, Bernardi L, Fagerudd J, Mantysaari M, Af Bjorkestén CG, Lindholm H *et al.* Early autonomic dysfunction in type 1 diabetes: a reversible disorder? *Diabetologia* 2009; **52**: 1164–1172.
- 15 Zobel EH, Winther SA, Hasbak P, von Scholten BJ, Holmvang L, Kjaer A *et al.* Myocardial flow reserve assessed by cardiac 82Rb positron emission tomography/computed tomography is associated with albumin excretion in patients with Type 1 diabetes. *Eur Heart J Cardiovasc Imaging* 2018; **20**: 796–803.
- 16 Bernardi L, Gordin D, Bordino M, Rosengard-Barlund M, Sandelin A, Forsblom C *et al.* Oxygen-induced impairment in arterial function is corrected by slow breathing in patients with type 1 diabetes. *Sci Rep* 2017; **7**: 6001.
- 17 Spallone V, Ziegler D, Freeman R, Bernardi L, Frontoni S, Pop-Busui R *et al.* Cardiovascular autonomic neuropathy in diabetes: clinical impact, assessment, diagnosis, and management. *Diabetes Metab Res Rev* 2011; **27**: 639–653.
- 18 Cardone C. I test che valutano la risposta riflessa cardiovascolare. *Neuropatia Diabetica: Rassegna Bibliografica* 1990; **2**: 151–160.
- 19 Spallone V, Bellavere F, Scionti L, Maule S, Quadri R, Bax G *et al.* Recommendations for the use of cardiovascular tests in diagnosing diabetic autonomic neuropathy. *Nutr Metab Cardiovasc Dis* 2011; **21**: 69–78.
- 20 Lefrandt JD, Hoogenberg K, van Roon AM, Dullaart RP, Gans RO, Smit AJ. Baroreflex sensitivity is depressed in microalbuminuric type I diabetic patients at rest and during sympathetic manoeuvres. *Diabetologia* 1999; **42**: 1345–1349.

- 21 Kaur M, Chandran DS, Jaryal AK, Bhowmik D, Agarwal SK, Deepak KK. Baroreflex dysfunction in chronic kidney disease. *World J Nephrol* 2016; **5**: 53–65.
- 22 Harris ND, Ibrahim S, Radatz M, Ward JD. Measurements of oxygen saturation in normal human sural nerve by micro-lightguide spectrophotometry and endoscopy. *Adv Exp Med Biol* 1997; **428**: 15–20.

FIGURE 1 Per cent change from baseline after interventions in all participants ($n = 54$). (a) Change in baroreflex sensitivity, (b) change in standard deviation of the normal to normal interval (SDNN; a measure of heart rate variability), (c) change in blood oxygen saturation and (d) change in arm oxygen saturation. Data are shown as per cent change from baseline to follow-up (after intervention) with 95% confidence intervals (bars). *P*-values for test of significance of the difference are from mixed-effects models with a patient-specific random intercept, with change in outcome variables as outcome and the three listed interventions as exposures. Baseline: paced breathing at a standardized rate (15 breath/min) in room air. (A) Acute oxygen inhalation: nasal inhalation of pure oxygen (6 l/min, 15 breath/min). (B) Slow deep breathing: six deep breaths per min in room air. (C) Combination: six deep breaths per min combined with nasal inhalation of pure oxygen.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Change in % from baseline after interventions in all participants ($n = 54$) with spontaneous breathing as baseline.

Table 1. Clinical characteristics of participants

	All (N = 54)	Normoalbuminuria (N = 29)	Macroalbuminuria (N = 25)	P-value
Gender (F : M)	23 : 31	12 : 27	11 : 14	0.846
Age (years)	60 ± 10	60 ± 9	60 ± 10	0.979
Duration of diabetes (years)	38 ± 14	33 ± 13	43 ± 15	0.015
BMI (kg/m ²)	26.5 ± 4.4	25.6 ± 4.2	27.4 ± 4.5	0.136
Current smokers	7 (13)	4 (14)	3 (13)	0.898
Alcohol (units per week)	7 [3, 15]	9 [4, 18]	7 [2, 14]	0.567
Blood oxygen saturation (%)	97.3 ± 1.8	97.3 ± 1.0	97.2 ± 2.4	0.753
Arm tissue oxygen saturation (%)	67.3 ± 2.3	67.4 ± 2.4	67.0 ± 2.1	0.344
Anti-hypertensive treatment	41 (76)	16 (55)	25 (100)	<0.001
Beta blockers	8 (15)	1 (3)	7 (28)	0.019
RAAS inhibitors	37 (69)	13 (45)	24 (96)	<0.001
HbA _{1c} (mmol/mol)	62 ± 10	62 ± 8	62 ± 11	0.902
HbA _{1c} (%)	7.8 ± 3.1	7.8 ± 2.9	7.8 ± 3.2	
UACR (mg/g creatinine)	8.5 [3.3, 116.7]	3.3 [2.7, 5.3]	125 [54.0, 300.0]	–
LDL cholesterol (mmol/l)	2.2 ± 0.7	2.3 ± 0.7	2.2 ± 0.7	0.693
eGFR (ml min ⁻¹ 1.73m ⁻²)	77 ± 23	89 ± 11	62 ± 24	–
SBP (mmHg)	135 ± 17	133 ± 15	138 ± 20	0.308
DBP (mmHg)	76 ± 10	79 ± 10	74 ± 10	0.078
Heart rate (bpm)	70 ± 12	70 ± 12	71 ± 11	0.569
CAN*	12 (22)	4 (7)	8 (15)	0.083
Elevated coronary calcium score (> 300)†	16 (30)	5 (9)	11 (20)	0.007
Baroreflex sensitivity (ms/mmHg)	4.8 [2.8, 7.7]	8.4 [4.9, 13.2]	4.4 [3.3, 5.9]	0.004
SDNN (ms)	9.5 [5.8, 15.9]	13.9 [7.1, 17.6]	7.8 [4.2, 13.9]	0.020

Data are presented as *n* (%), mean ± SD and median [IQR]. Categorical data were analysed with χ^2 test and Fisher's

exact test when appropriate. Quantitative data were analysed with *t*-test.

Oxygen saturation levels, baroreflex sensitivity and standard deviation of the normal to normal interval (SDNN) are measured in supine position with paced breathing at a standardized rate (15 breaths/min). Normoalbuminuria was defined as urine albumin < 30 mg/24 h or < 30 mg/g creatinine in two of three consecutive measurements and macroalbuminuria was defined as urine albumin > 300 mg/24 h or > 300 mg/g creatinine in two of three consecutive measurements.

*Only 43 participants were evaluated for cardiovascular autonomic neuropathy (CAN), the remaining 11 participants had poor data quality.

†Only 48 participants showed up for the measurement.

RAAS, renin–angiotensin aldosterone system; UACR, urine albumin to creatinine ratio.

Table 2. Per cent Change from baseline after interventions in all participants (*n* = 54) with paced breathing as baseline

Interventions	Description	Primary outcomes		Secondary outcomes	
		Baroreflex sensitivity	SDNN (ms)	Blood oxygen saturation	Arm oxygen saturation (%)
		(ms/mmHg)		(%)	
A: acute oxygen inhalation	Baseline	4.7 (3.9, 5.7)	10.3 (8.3, 12.9)	97.2 (96.7, 97.7)	67.2 (66.6, 67.8)
	Follow-up	5.8 (4.7, 7.0)	11.2 (9.0, 14.0)	98.2 (98.1, 98.4)	67.9 (67.3, 68.5)
	Difference (%)	21 (10, 34)	8 (-1, 17)	1.0 (0.5, 1.4)	1.0 (0.4, 1.6)
	<i>P</i> -value vs. baseline	<0.001	0.056	<0.001	0.002
B: slow deep breathing	Follow-up	6.2 (5.0, 7.7)	13.7 (10.8, 17.4)	97.3 (96.8, 97.7)	67.1 (66.4, 67.7)
	Difference (%)	32 (13, 53)	33 (18, 49)	0.0 (-0.2, 0.2)	0.0 (-0.7, 0.2)
	<i>P</i> -value vs. baseline	<0.001	<0.001	0.827	0.263
C: Combination (A+B)	Follow-up	6.1 (5.0, 7.5)	14.9 (11.7, 19.0)	98.3 (98.2, 98.5)	67.7 (67.1, 68.2)
	Difference (%)	30 (10, 54)	44 (27, 64)	1.0 (0.7, 1.6)	0.7 (0.1, 1.2)
	<i>P</i> -value vs. baseline	0.004	<0.001	<0.001	0.021
Comparisons					
B vs. A	<i>P</i> -value B vs. A	0.507	<0.001	<0.001	<0.001
C vs. A	<i>P</i> -value C vs. A	0.335	<0.001	0.010	0.026
C vs. B	<i>P</i> -value C vs. B	0.837	0.075	<0.001	0.001

Data for baseline and follow-up levels are presented as geometric means (95% confidence intervals). *P*-values for test of significance of the difference are from mixed effects models with a patient-specific random intercept, with change in outcome-variables as outcome and the three listed interventions as exposures.

Baseline: Paced breathing at a standardized rate (15 breath/min) in room air.

A: Acute oxygen inhalation is nasal inhalation of pure oxygen (6 l/min, 15 breath/min). B: Slow deep breathing is six deep breaths per min in room air. C: Combination is six deep breaths per min combined with nasal inhalation of pure oxygen.

SDNN, Standard deviation of the normal to normal interval (a measure of heart rate variability).

Comparisons are *P*-values for the pairwise tests of difference in change from baseline between the intervention groups.

Figure 1 Change in % from baseline after interventions in all participants (n=54)

Data are % change from baseline to follow up (after intervention) with 95% confidence intervals. P-values for test of significance of the difference are from mixed effects models with a patient-specific random intercept, with change in outcome-variables as outcome and the three listed interventions as exposures.

Panel (a) is change in baroreflex sensitivity, panel (b) is change in SDNN, panel (c) is change in blood oxygen saturation and panel (d) is change in arm oxygen saturation.

Baseline: Paced breathing at a standardized rate (15 breaths per minute) in room air.

Interventions: A: "Acute oxygen inhalation" is nasal inhalation of pure oxygen (6 L per minute, 15 breaths per minute). B: "Slow deep breathing" is six deep breaths per minute in room air.

C: "Combination" is six deep breaths per minute combined with nasal inhalation of pure oxygen. SDNN = Standard deviation of the normal to normal interval (a measure of heart rate variability).