

# Effects of a Single Bout of Interval Hypoxia on Cardiorespiratory Control and Blood Glucose in Patients With Type 2 Diabetes

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**OBJECTIVE**—Hypoxia may cause functional autonomic imbalance in diabetes. Intermittent hypoxia (IH), a technique improving the adaptation to hypoxia, might improve cardiorespiratory reflexes and, ultimately, blood glucose concentrations in patients with type 2 diabetes. We tested whether a single bout of IH could initiate a long-lasting response potentially leading to better adaptation to hypoxia.

**RESEARCH DESIGN AND METHODS**—In 14 patients with type 2 diabetes without autonomic complications, we measured blood pressure, heart rate, oxygen saturation, chemo-reflex (hypoxic and hypercapnic ventilatory responses, ventilatory recruitment threshold), and baroreflex sensitivity before, immediately after, and 3 and 6 h after a 1-h single bout of IH (6-min breathing of 13% oxygen mixture 5 times each separated by 6-min recovery). The measurements were repeated on a placebo day (at least 1 week apart, in random sequence) when subjects were only breathing room air (single-blind protocol).

**RESULTS**—IH significantly increased hypercapnic ventilatory responses and reduced ventilatory recruitment threshold, and increased oxygen saturation and blood pressures, whereas increases in heart rate variability and baroreflex sensitivity were not significant. Blood glucose significantly decreased after IH. No such changes were observed during the placebo day, except an increase in oxygen saturation. Some of the effects lasted 3 h after IH, and some even persisted until 6 h after IH.

**CONCLUSIONS**—A single bout of IH induced an initial adaptation to hypoxia, with improvement in cardiorespiratory reflexes and reduction in blood glucose. Patients with type 2 diabetes could potentially benefit from the application of a full (>2 weeks) IH intervention.

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In diabetes, abnormalities of the autonomic nervous system (ANS) represent one important complication of the disease (1) because it can predispose to severe cardiovascular events (2,3). ANS dysfunction is not exclusively induced by anatomic lesions but has an important functional component (4). Low oxygen content (hypoxia), described in most

organs and tissues of diabetic patients (5–9), recently has been suggested as one cause of ANS abnormalities (10,11). As a consequence, improvement of existing hypoxia might improve autonomic abnormalities that, in turn, also might have consequences on glucose metabolism.

One possible strategy to improve hypoxia could be the application of intermittent

hypoxia (IH). IH improves exercise capacity in athletes, improves the acclimatization to high altitude in climbers (12,13), and improves ANS in various patients (14,15). The technique consists of intermittent exposures to hypoxic stimuli (3–5 times per day, lasting at least 5–6 min, and spaced at least by 5–6 min) repeated over 2–3 weeks. The principle of the method is like any other type of training: a given stress (here, hypoxia), if appropriately administered and spaced in time, creates a counter-regulatory response that lasts longer and, when repeated a sufficient number of times, leads to a sustained “training” effect (16). IH could increase resting oxygen saturation by increasing the ventilation and the chemoreflexes and, consequently, could reduce the sympathetic activation associated with hypoxia, as previously shown in patients with chronic bronchitis (17).

However, until now the effects of IH in patients with type 2 diabetes are unknown, even though respiratory and cardiovascular reflexes (18–24) and molecular responses to hypoxia (25) have been found to be generally impaired. Therefore, performing a short course of IH might initiate a chain of events that may eventually lead to an “acclimatization” process (when prolonging IH to >1 day). The consequence of relieving hypoxia should be restoration and correction of the cardiorespiratory reflexes.

If positive results could be found from this initial study performed in type 2 diabetic subjects without complications, then performing a full training period of IH could be justified in diabetes to test whether this intervention is able to prevent the development of diabetes complications.

## RESEARCH DESIGN AND METHODS

### Participants

In this single-blind, placebo-controlled study, we tested 14 type 2 diabetic subjects

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(3 female, 11 male) without clinical evidence of respiratory dysfunction or autonomic complications. Patients were recruited through general practitioners in and around Innsbruck, Austria. Exclusion criteria were the presence of exercise-limiting pulmonary or musculoskeletal diseases, unstable diabetes, previous or acute myocardial infarction, proliferative retinopathy, cardiovascular complications, ventricular arrhythmias and atrial fibrillation, severe hypertension ( $\geq 180/110$  mmHg), unstable or stable angina, smoking, insulin treatment, and treatment with  $\beta$ -blockers. Participants were advised to maintain their habits concerning medications, nutrition, and extent of physical activity. The conditions mentioned in the exclusion criteria were assessed using a medical interview. In addition, the participants

underwent clinical examination, including blood pressure measurements, determination of red and white blood cell counts, and lung function testing. A stress test (i.e., spiroergometry including electrocardiogram monitoring) was performed to determine exercise capacity ( $VO_{2peak}$ ) and to reveal ischemic heart disease.

The study was accomplished in accordance with the Declaration of Helsinki and was approved by the Ethics Committee of the Medical University of Innsbruck. Written informed consent was obtained before the study from each subject. Characteristics of the study groups are shown in Table 1.

### Protocol

To test this hypothesis, we exposed patients with type 2 diabetes to 1 h of IH and

followed the response over the course of 6 h thereafter in a single-blind, placebo-controlled protocol. All subjects were examined on 2 different days with a minimum time interval of 3–5 days to avoid possible carryover effects. Baseline measurements on each day were performed during the morning at least 2 h after breakfast and subjects were advised to abstain from caffeinated beverages for 12 h and from alcohol for 36 h before the examinations. The intermittent hypoxic protocol was applied on one day (IH day), whereas on the other day the same participants underwent an identical protocol of solely breathing room air (placebo day). Thus, subjects served as their own controls (crossover design). The sequence of hypoxia day or placebo day was randomized to avoid possible confounding factors attributable to the “learning” effect. After the baseline measurements, the hypoxic or placebo sessions were performed during 1 h in the morning under standardized conditions (Supplementary Fig. 1). Each hypoxia session had five hypoxic periods (13%  $O_2$  inspired fraction of oxygen) each lasting 6 min, interspersed with five normoxic exposures of the same duration. The procedure for the normoxia day was the same, but the participants inhaled normoxic air. Patients were breathing hypoxic or normoxic air in a supine position using a facial mask. Breathing periods were regulated and controlled by a technician under the supervision of a medical doctor, and without being noticed by the patient. After the hypoxic or placebo exposure, three further structured measurement sessions were performed immediately after, after 3 h, and after 6 h. During the exposure, blood pressure (Portapres; FMS Medical Systems, Amsterdam, the Netherlands), heart rate, and arterial oxygen saturation (COSMOplus; Novamatrix) were continuously monitored. The appearance of symptoms or a decline of oxygen saturation  $<80\%$  was set as criteria for interrupting the administration of hypoxia until saturation levels recovered to  $\geq 80\%$ . After measurements immediately after IH, a meal was given to the patients, based on their specific diet requirement. The same meal was administered to each patient on both the hypoxia and the placebo days.

### Cardiovascular and respiratory testing

To examine the control of the respiratory systems, measurements of hypoxic ventilatory response (HVR) and hypercapnic ventilatory response (HCVR) were performed four times per day (before,

**Table 1—Baseline characteristics of type 2 diabetic patients**

Gender (female/male)	3/11
Age (years)	59.3 $\pm$ 1.5
Height (cm)	174.9 $\pm$ 1.5
Weight (kg)	90.3 $\pm$ 3.9
BMI (kg/m <sup>2</sup> )	29.4 $\pm$ 1.0
Systolic blood pressure (mmHg)	130.7 $\pm$ 2.2
Diastolic blood pressure (mmHg)	78.9 $\pm$ 1.3
Hypertensive subjects (n)	6
Resting heart rate (beat/min)	80.3 $\pm$ 3.6
Waist/hip ratio	1.05 $\pm$ 0.3
Serum creatinine ( $\mu$ mol/L)	82.4 $\pm$ 4.2
Fasting plasma glucose (mmol/L)	7.7 $\pm$ 0.6
HbA <sub>1c</sub> (%)	7.2 $\pm$ 0.3
Total cholesterol (mmol/L)	4.6 $\pm$ 0.2
HOMA-IR	7.6 $\pm$ 1.7
$VO_{2peak}$ (mL/kg/min)	28.2 $\pm$ 1.3
Physical activity (h/week)	4.39 $\pm$ 0.9
HDL cholesterol (mmol/L)	1.2 $\pm$ 0.08
Triglycerides (mmol/L)	1.7 $\pm$ 0.2
Microalbuminuria (n; %)	1; 7.1
Macroalbuminuria (n)	0
GFR (mL/min)	113.3 $\pm$ 10.0
Baseline $O_2$ saturation (%)	94.8 $\pm$ 0.4
Deep breathing (E:I ratio)	1.16 $\pm$ 0.02
Smokers (n)	0
Duration of diabetes (years)	9.8 $\pm$ 1.8
FEV <sub>1</sub> (L)	3.62 $\pm$ 0.2
FEVC (L)	4.02 $\pm$ 0.2
FEV <sub>1</sub> /FEVC (%)	90.4 $\pm$ 3.1
Medications	
Metformin (n)	7
Sulphonylureas (n)	4
Dipeptidyl peptidase-4 inhibitors (n)	5
Antihypertensive medications (n)	4

Data are presented as means  $\pm$  SEM. E:I ratio, expiration/inspiration ratio; FEV<sub>1</sub>, forced expiratory volume in 1 s; FEVC, forced expiratory vital capacity; GFR, glomerular filtration rate; HOMA-IR, homeostasis model assessment of insulin resistance.

immediately after, and 3 and 6 h after a 1-h single bout of IH) during IH and placebo day. All participants were evaluated in a lying position in a silent and well-tempered room. At first, 4-min recordings at rest were obtained during spontaneous breathing for the measurement of baroreflex sensitivity (BRS). Then, using a mouthpiece and an antibacterial filter, they were connected to a rebreathing circuit, as previously described and validated (17,26–28). During the testing, we continuously measured end-tidal CO<sub>2</sub> (CO<sub>2</sub>-et) applying a capnograph connected to the mouthpiece (COSMOplus) and oxygen saturation (SaO<sub>2</sub>). Continuous airway flow measurements were arranged through a heated Fleish pneumotachograph (Metabo, Epalinges, Switzerland) that was connected to a differential pressure transducer (RS part N395–257; Corby) connected in series to the expiratory component of the rebreathing system. The electrocardiogram was recorded via chest leads, whereas the continuous blood pressure was recorded using a digital plethysmograph (Portapres).

Ventilation is stimulated when inhaled oxygen is progressively reduced or carbon dioxide concentration increases. To test the response to hypoxia, a variable part of the exhausted air was passed into a scrubbing circuit filled with soda lime, subsequently returning it into the rebreathing bag. This enabled a permanent adjustment of the CO<sub>2</sub>-et values to maintain the values at a constant level. When the percentage of arterial SaO<sub>2</sub> reached 80%, the examination of HVR was terminated. The response to hypercapnia was evaluated by continuously supplying oxygen at very low flow to maintain the SaO<sub>2</sub> percentage at constant levels (>98%). At the same time, bypassing the reservoir with soda lime, the exhaled air directly arrived in the rebreathing bag, resulting in CO<sub>2</sub>-et values progressively increasing. The test was completed when CO<sub>2</sub>-et increased 13 mmHg above resting levels. Resting data were collected before each rebreathing test during 4 min of spontaneous breathing of room air.

#### Data acquisition and analysis

All signals were continuously acquired on a personal computer (Apple Macintosh, Cupertino, CA) at 600 samples per channel. An automatic and interactive program, written in BASIC by one of our group members (L.B.), was implemented to identify each single breath and to obtain

breath-by-breath minute ventilation, tidal volume by integration of the flow signal, and, in addition to breathing rate, CO<sub>2</sub>-et and SaO<sub>2</sub> percentage.

#### Measurement of chemoreflex sensitivity

The slope of the linear regression line of minute ventilation versus SaO<sub>2</sub> or CO<sub>2</sub>-et indicates the chemoreflex sensitivity to hypoxia or hypercapnia, respectively. The change in ventilation attributable to hypercapnia is interpreted as a main indicator for the central chemoreflex sensitivity, whereas the change in ventilation attributable to hypoxia is interpreted as a main indicator for peripheral chemoreflex sensitivity. The point at which the ventilation started to increase during the progressive HCVR (called ventilatory recruitment threshold [VRT-CO<sub>2</sub>]; Supplementary Fig. 2) was identified by interpolating the ventilation/CO<sub>2</sub>-et plot using a fourth-order polynomial function.

#### Assessment of baroreflex sensitivity

The BRS was measured during recordings of spontaneous breathing at each measurement session. Previous studies have shown a poor correlation between different indices of BRS without better performance of one over the others (29). Accordingly, BRS was calculated as the average of the following seven different methods (30): positive and negative sequence methods (31); the  $\alpha$  coefficient in the low- and high-frequency bands and its average (32); the transfer function technique (33); and the ratio of SDs of R-R interval and systolic blood pressure variability. In addition to the BRS, a global index of heart rate variability was assessed using the SD of the R-R interval (SDNN), because this variable has a more normal distribution as compared with other indices of variability (e.g., variance).

#### Blood analyses

Venous blood samples were taken to analyze total serum cholesterol, HDL, and hemoglobin. To determine fasting plasma glucose, HbA<sub>1c</sub>, creatinine, and triglycerides, capillary blood sampling was performed after a 10-h overnight fasting using heparinized glass capillaries. Blood analyses were accomplished by standard local devices (Reflotron sprint; Boehringer Mannheim; Miniphotometer LP 20, Dr. Lange). Glomerular filtration rate was calculated by the Cockcroft-Gault formula. Nonfasting plasma glucose levels before and after the hypoxic or normoxic

exposures were estimated taking capillary blood samples (Multicare In-Vitro Diagnostic System; Biochemical System).

#### Urine samples

First morning urine samples were collected for determination of microalbuminuria (Micral-Test; Roche Diagnostics GmbH) and macroalbuminuria (Combur-Test; Roche Diagnostics GmbH).

#### Statistical analysis

Data are presented as means  $\pm$  SEM. Data analysis was performed applying the SPSS statistical software package 18.  $P \leq 0.05$  (two-tailed) was considered as statistically significant. Comparisons were performed between the same time points of the hypoxia and placebo days and, within each day, by comparing the differences from the first measurement (baseline) of each day by paired  $t$  test. In addition, the differences with respect to baseline in the hypoxia and placebo days were compared by paired  $t$  test to assess whether the intermittent hypoxia induced different trends during the 2 days.

**RESULTS**—The hypoxic exposure and the evaluation of respiratory function were well-tolerated by each participant and adverse events did not appear. Resting respiratory data and the complete results of the cardiovascular, respiratory, and metabolic variables are described in Table 2.

#### Baseline respiratory and cardiovascular data

There were no significant differences in any of the measured variables at baseline on the placebo day or the IH day. In general, respiratory reflexes and BRS appeared slightly reduced as compared with our reference database (17).

#### Effects of interval hypoxia

**Effects of IH on respiratory data.** HCVR significantly increased after IH (Fig. 1) and tended to remain elevated (3 h after IH:  $P = 0.096$ ; 6 h after IH:  $P = 0.105$ ) thereafter. In contrast, an opposite trend could be observed immediately after the placebo exposure, which, however, was no more apparent at 3 h or 6 h after exposure. Additionally, the VRT-CO<sub>2</sub> was reduced immediately after the hypoxia exposure, whereas on the placebo day VRT-CO<sub>2</sub> did not decrease (Fig. 1). As a consequence, the differences from baseline were significant not only immediately after the intervention but also 3 h later. The HVR

Table 2—Cardiorespiratory and metabolic data

	Baseline (t1)	Post (t2)	Post II (t3)	Post III (t4)
HCVR (Lmin <sup>-1</sup> /mmHg CO <sub>2</sub> -et)				
IH	0.75 ± 0.11	0.88 ± 0.13*§	0.92 ± 0.12	0.90 ± 0.15
Placebo	0.73 ± 0.14	0.65 ± 0.13	0.82 ± 0.13	0.83 ± 0.11
HVR (Lmin <sup>-1</sup> % SaO <sub>2</sub> )				
IH	-0.49 ± 0.14	-0.53 ± 0.15	-0.62 ± 0.18	-0.69 ± 0.22
Placebo	-0.40 ± 0.13	-0.45 ± 0.10	-0.48 ± 0.19	-0.54 ± 0.13
VRT-CO <sub>2</sub> (mmHg)				
IH	41.6 ± 0.7	41.0 ± 0.7*‡	42.0 ± 0.9†	42.1 ± 0.9
Placebo	41.0 ± 0.5	41.4 ± 0.5	42.4 ± 0.7§	42.3 ± 0.6
R-R (ms)				
IH	902.1 ± 33.4	996.1 ± 32.6¶	901.9 ± 40.1	889.3 ± 45.3
Placebo	920.1 ± 33.2	982.3 ± 33.0§	856.6 ± 38.0§	890.2 ± 34.0
SDNN (ms)				
IH	29.6 ± 2.7	49.0 ± 9.0	33.6 ± 3.7	30.6 ± 4.3
Placebo	32.5 ± 4.1	41.3 ± 4.4	26.9 ± 3.7	28.9 ± 3.3
SBP (mmHg)				
IH	120.5 ± 3.8	138.2 ± 5.6*§	126.3 ± 5.0†‡	131.3 ± 5.7
Placebo	126.3 ± 3.9	138.0 ± 5.5	120.1 ± 6.1	127.5 ± 5.3
DBP (mmHg)				
IH	59.9 ± 2.3	67.6 ± 2.9‡	62.2 ± 2.7*	64.8 ± 2.9‡
Placebo	63.9 ± 3.1	68.8 ± 2.7	58.5 ± 3.2‡	63.3 ± 2.7
SaO <sub>2</sub> (%)				
IH	94.8 ± 0.4	95.9 ± 0.3‡	95.6 ± 0.3‡	95.0 ± 0.7
Placebo	94.6 ± 0.4	95.7 ± 0.2‡	95.3 ± 0.3	95.0 ± 0.9
BRS (ms/mmHg)				
IH	7.3 ± 0.8	10.2 ± 1.5	10.2 ± 3.4	9.5 ± 2.2
Placebo	8.3 ± 1.5	8.2 ± 1.3	5.7 ± 0.6	6.6 ± 0.7
Respiration rate (breaths/min)				
IH	12.4 ± 0.7	12.3 ± 0.9	13.1 ± 0.8	12.4 ± 1.0
Placebo	12.2 ± 0.9	11.7 ± 1.0	12.6 ± 0.8	13.2 ± 1.0
VE (L/min)				
IH	10.2 ± 1.3	10.9 ± 1.2	11.2 ± 1.0	11.9 ± 1.5‡
Placebo	10.5 ± 1.3	10.1 ± 1.3	10.7 ± 1.2	10.9 ± 0.9
Vt (L)				
IH	866.7 ± 101.0	938.0 ± 99.3	911.2 ± 97.8	1,030.8 ± 124.9*‡
Placebo	950.7 ± 123.9	919.2 ± 89.8	896.5 ± 103.4	928.5 ± 118.7
CO <sub>2</sub> -et (mmHg)				
IH	35.2 ± 1.3	37.0 ± 1.6§	39.6 ± 0.9¶	38.6 ± 1.2¶
Placebo	34.1 ± 1.2	37.5 ± 1.2§	39.1 ± 1.1¶	39.2 ± 1.1¶
Blood glucose (mmol/L)				
IH	8.3 ± 0.9	6.2 ± 0.6*§	9.0 ± 0.8	7.0 ± 0.7*
Placebo	7.1 ± 0.8	6.3 ± 0.5	9.4 ± 0.8§	7.6 ± 0.8

Data are mean ± SEM. DBP, diastolic blood pressure; SBP, systolic blood pressure; t1, baseline measurements; t2, measurements immediately after IH; t3, measurements 3 h after IH; t4, measurements 6 h after IH; VE, minute ventilation; Vt, tidal volume. \**P* < 0.05; †*P* < 0.01 comparing Δ(t - t1) IH vs. Δ(t - t1) placebo day; ‡*P* < 0.05; §*P* < 0.01; ¶*P* < 0.001 compared with t1 of same day.

showed no significant changes after IH (Fig. 1). After both placebo and IH, CO<sub>2</sub>-et increased with respect to baseline. Slight increases in tidal volume and minute ventilation were observed 6 h after IH, whereas respiration rate did not change.

Oxygen saturation increased significantly after both IH and placebo; however, the extent of the increase was not significantly higher after IH than after placebo.

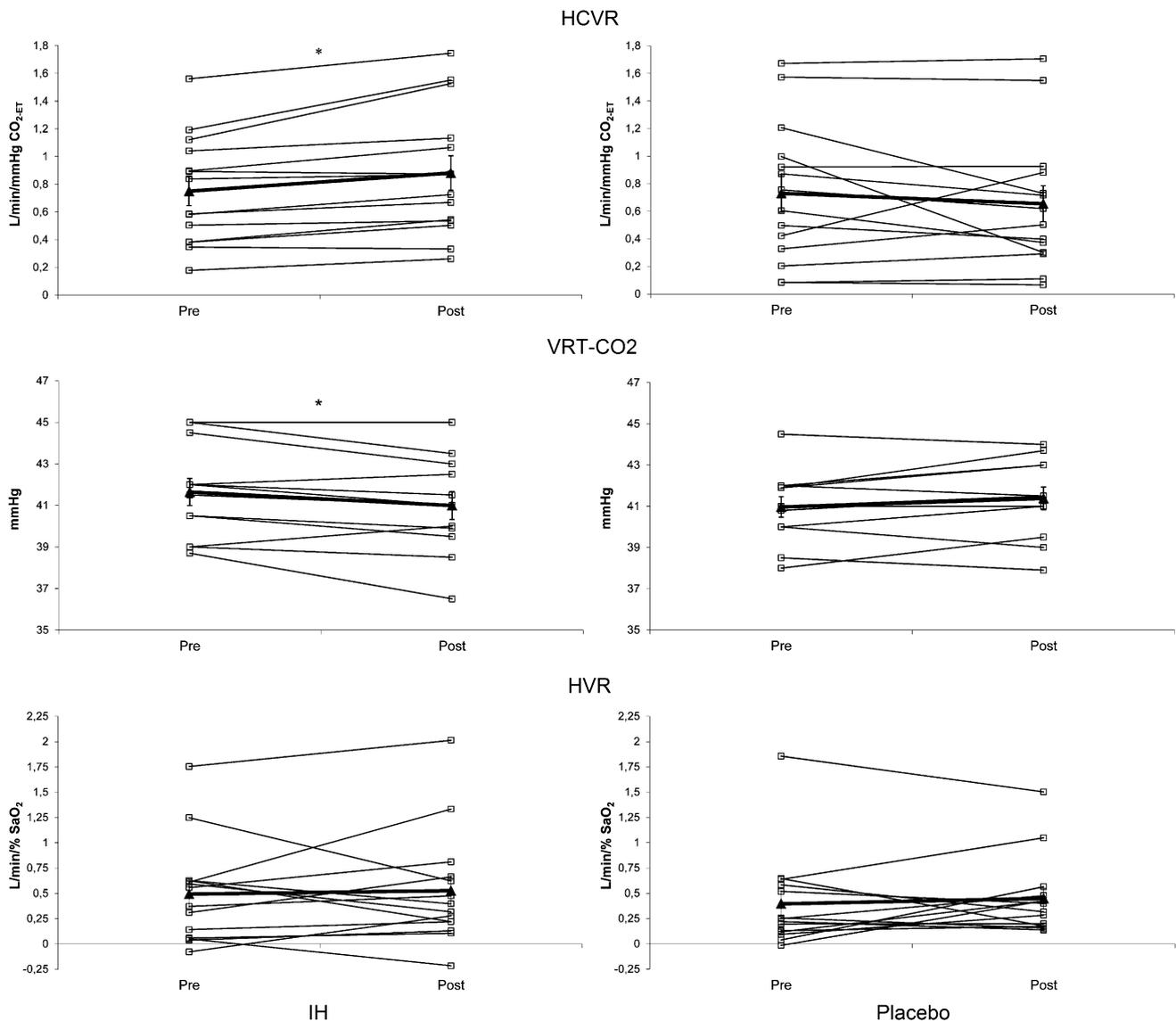
#### Effects of IH on cardiovascular data.

The R-R interval significantly increased immediately after the exposure during both testing days. Thereafter, R-R interval decreased more on the placebo day than after IH. Heart rate variability (SDNN) increased, although not significantly (*P* = 0.059). Systolic and diastolic blood pressures significantly increased subsequent to the hypoxia exposure, but decreased again at 3 h and 6 h from baseline.

Changes in baroreflex sensitivity were not significantly different between the IH day and the placebo day.

#### Changes in blood glucose

Baseline blood glucose concentrations before the chemoreflex testing did not differ between the intervention day and the placebo day (*P* = 0.12). A significant decrease in plasma glucose (*P* < 0.01) occurred after the hypoxic exposure, whereas



**Figure 1**—Plot shows changes in HCVr (top panel), VRT- $\text{CO}_2$  (middle panel), and HVR (bottom panel) immediately after 1 h of IH or placebo exposure ( $n = 14$ ). \*Significant differences ( $P < 0.05$ , paired  $t$  test) from baseline ( $t_1$ ) to postintervention ( $t_2$ ). Bold lines show mean values  $\pm$  SEM.

no significant change was apparent on the placebo day (Fig. 2A). During the second evaluation after the intervention, the glucose concentrations increased during both days because of the effect of the meal; however, when evaluated in terms of differences from the baseline values a clear trend emerged, showing a progressive decrease in blood glucose after the IH as compared with the corresponding times of the placebo day (Fig. 2B).

To our knowledge, this is the first study specifically evaluating the cardiovascular and respiratory effects of IH in patients with type 2 diabetes without autonomic complications. The main findings of the present investigation, limited to a single bout of IH, are of potential practical

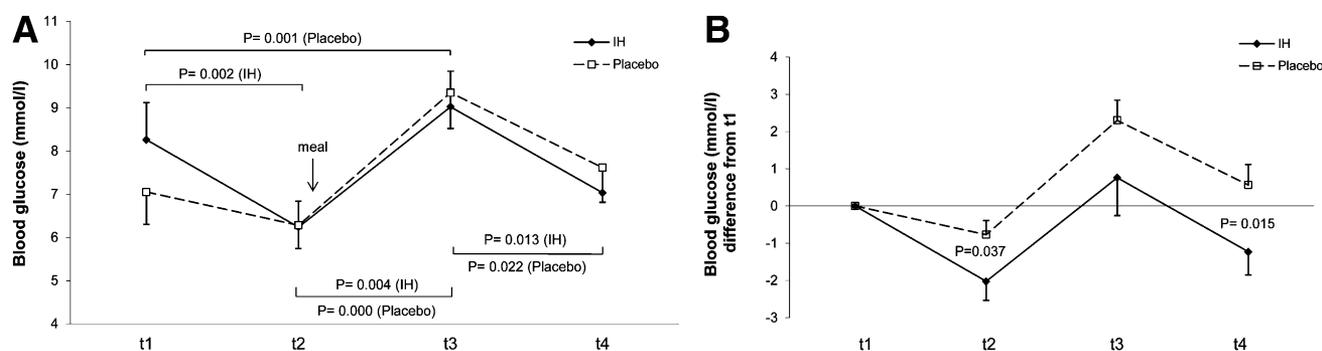
interest. Exposure to IH rebalanced the main cardiorespiratory reflexes, accompanied by a sustained reduction in blood glucose after IH.

#### Effects of IH on respiratory control in type 2 diabetes

A decreased ventilatory response to hypercapnia (20–22) or hypoxia (18–20) previously has been described in diabetes, and this impaired ventilatory response may increase the risk of diabetes complications. Likewise, in our study, baseline respiratory reflexes appeared slightly decreased as compared with our reference values (17). In line with these findings, VRT- $\text{CO}_2$  was shifted to the right at baseline, indicating that these patients need

to reach a higher level of  $\text{CO}_2$  before starting ventilation. After IH, we observed a significant increase in HCVr and a shift to the left in VRT- $\text{CO}_2$ . No change was observed in HVR. IH caused a slight increase in tidal volume and in minute ventilation.

The evident increase in the HCVr and the lack of change in the HVR seems, at first sight, paradoxical. However, the first effect of an increase in ventilation is a reduction in the  $\text{CO}_2$ -et. This limits or even stops the hyperventilation, because the carbon dioxide is a potent stimulus of ventilation. Accordingly, the only possibility to allow a persistent hyperventilation is through resetting of the VRT- $\text{CO}_2$  at lower levels. The changes in HCVr and VRT- $\text{CO}_2$  thus are compatible with the



**Figure 2**—Blood glucose changes over time on the hypoxia day (continuous line) and the placebo day (dotted line). Measurements were performed at baseline (t1), immediately after (t2), 3 h after (t3), and 6 h after (t4) the hypoxic or placebo exposure (n = 14). Standardized meal was taken after t2 on both days. A: Comparing blood glucose levels with t1 of the same day. B: Comparing  $\Delta$  (t – t1) IH vs.  $\Delta$  (t – t1) placebo day. Data are presented as means  $\pm$  SEM.

initial chain of events leading to a training effect on the cardiorespiratory reflexes. The oxygen saturation increased significantly after the IH, but also during the placebo day. Because these changes did not seem to result from an increase in ventilation, alternative explanations could be an improvement in gas exchange (8) or a favorable change in the oxygen transport of hemoglobin (34). The increase in oxygen saturation during placebo might suggest that the exposure to hypoxia during the measurement of the HVR could have modified some variables also during the placebo day.

### Effects of IH on cardiovascular control

Abnormalities of the cardiovascular control, evidenced by decreased BRS and heart rate variability, have been observed in patients with type 2 diabetes (23,24). Impairment of cardiovascular control can lead to severe multiorgan dysfunction and, finally, to adverse cardiovascular events in people with diabetes (2,3). Immediately after IH, a significant increase in the R-R interval was found, together with a nonsignificant increase in BRS and SDNN, suggesting an increase in parasympathetic activity. Increases in systolic and diastolic blood pressures also were evident after IH, and they could be explained by the increased oxygenation of the hypoxic patients. In a previous article (10), we showed that patients with type 1 diabetes had increased blood pressure in response to hyperoxia. This might have stimulated the increase in parasympathetic activity that prolonged the R-R interval (i.e., induced a relative bradycardia) and increased the SDNN and the BRS (similar to what found in the current study). However, Haider et al. (17) showed that the long-term effect

of IH (1 h per day for 3 weeks) in chronic obstructive pulmonary disease patients was even a slight decrease in systolic blood pressure. Thus, one may suggest that the increase in systolic blood pressure observed in our study may be an initial response that is followed by adaptation.

### Changes in blood glucose

Surprisingly, a significant decrease in blood glucose appeared. The link between hypoxia, cardiorespiratory reflexes, and blood glucose might be highly complex and will certainly require further studies. As speculation, this first observation that IH improves both cardiorespiratory reflexes and glucose concentrations could be the consequence of improving hypoxia through improved tissue oxygenation and reduced sympathetic activity. This may lead to better glucose utilization.

### Preexisting hypoxia in type 2 diabetic patients

Hypoxia is a new, yet common, finding in diabetes and may result from various factors. Diabetic patients show decreased skin oxygenation (reduced transcutaneous partial pressure of oxygen) and increased venous oxygen partial pressure (5). Glycosylation of hemoglobin decreases oxygen transport (6), whereas in the lungs the glycosylation of basal membranes leads to impaired diffusing capacity for carbon monoxide (7). Obstructive sleep apnea is another cause of hypoxia, particularly in patients with type 2 diabetes (9). In addition, low SaO<sub>2</sub> at rest has been observed in diabetic subjects (11). In contrast to healthy subjects, responses to experimental (in isolated cell and tissues) and clinical hypoxia in diabetes are impaired (25,35), possibly leading to diabetes complications.

### Limitations

In this investigation, the control day included a minor exposure to IH because each HVR test (which was repeated four times on IH days and placebo days) exposes the subject to a transitory hypoxia. In addition, some of the effects observed in this study might be mediated by hyperoxia (a small amount of oxygen had to be administered to perform the HCVR test). For safety reasons, because of the novelty of this approach, the subjects in this first study were a selected group of patients without evidence of autonomic complications. Therefore, the present findings may not be generalized or transferred to patients with diabetes with evidence of ANS abnormality.

**CONCLUSIONS**—Exposure to a single bout of IH improved hypoxia, rebalanced cardiorespiratory reflexes, and reduced glucose levels in patients with type 2 diabetes without autonomic complications. These preliminary findings support future studies applying IH for a longer period of time to test whether a full IH protocol may potentiate or prolong these initial positive effects, which potentially can have an important clinical impact.

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T.D. performed the experiments, researched data, and had the primary responsibility of drafting the manuscript. H.G. contributed to data collection and interpretation. P.-H.G. critically revised the manuscript for important intellectual content. M.B. supervised the

study, contributed to data collection, interpretation, and analysis, and edited the manuscript. L.B. developed the study concept and design, contributed to interpretation and analysis, edited the manuscript, and revised the manuscript. T.D. 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.

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