Effects of a Single Bout of Interval Hypoxia on Cardiorespiratory Control in Patients With Type 1 Diabetes

Tobias Duennwald,[1] Luciano Bernardi,[2,3,4] Daniel Gordin,[2,3] Anna Sandelin,[2] Anna Syreeni,[2] Christopher Fogarty,[2] Janne P. Kytö,[2] Hannes Gatterer,[1] Markku Lehto,[2] Sohvi Hörkkö,[5] Carol Forsblom,[2] Martin Burtscher,[1] and Per-Henrik Groop,[2,3,6] on behalf of the FinnDiane Study Group

Hypoxemia is common in diabetes, and reflex responses to hypoxia are blunted. These abnormalities could lead to cardiovascular/renal complications. Interval hypoxia (IH) (5–6 short periods of hypoxia each day over 1–3 weeks) was successfully used to improve the adaptation to hypoxia in patients with chronic obstructive pulmonary disease. We tested whether IH over 1 day could initiate a long-lasting response potentially leading to better adaptation to hypoxia. In 15 patients with type 1 diabetes, we measured hypoxic and hypercapnic ventilatory responses (HCVRs), ventilatory recruitment threshold (VRT-CO_2), baroreflex sensitivity (BRS), blood pressure, and blood lactate before and after 0, 3, and 6 h of a 1-h single bout of IH. All measurements were repeated on a placebo day (single-blind protocol, randomized sequence). After IH (immediately and after 3 h), hypoxic and HCVR increased, whereas the VRT-CO_2 dropped. No such changes were observed on the placebo day. Systolic and diastolic blood pressure increased, whereas blood lactate decreased after IH. Despite exposure to hypoxia, BRS remained unchanged. Repeated exposures to hypoxia over 1 day induced an initial adaptation to hypoxia, with improvement in respiratory reflexes. Prolonging the exposure to IH (>2 weeks) in type 1 diabetic patients will be a matter for further studies.
***Diabetes* 62:4220–4227, 2013**

Diabetes is closely related to impaired function of the autonomic nervous system (ANS). As a consequence, autonomic dysfunction can worsen the prognosis of the disease and result in serious complications (1,2).

Assuming that ANS abnormalities such as sympathetic overactivity or reduced cardiorespiratory reflexes might at an early stage be attributed to a functional origin (as they can be favorably influenced by simple functional maneuvers) rather than organic lesions (3), they could possibly be reversible by an appropriate intervention. In diabetic patients, low oxygen content (hypoxemia) is common in most organ and tissues (4–8). Hypoxia in the blood or tissues is known to induce sympathetic activation, hence, altering cardiovascular reflex tests regardless of neural damage (9,10).

Interval hypoxia (IH) could be a useful strategy to improve hypoxia, since IH has largely been implemented in the adaptation to high altitude (11,12). Due to the improved adaptation to hypoxia, IH also improves exercise performance in athletes (13) and improves ANS function in various diseases (14,15). IH consists of repeated short periods of hypoxia (5–6 min) interspersed by equal periods of normoxia, thus creating a sort of stress that in turn evokes a counterregulatory response by altering the preexisting homeostasis. If adequately administered, sufficient repetitions lead to a persisting supercompensatory ("training") effect (16,17) and improved response to hypoxia. The use of IH in patients with chronic bronchitis (18) increased ventilation, oxygen saturation, and chemoreflex activity; reduced hypoxia-dependent sympathetic overactivity; and right shifted the lactate-load curve during exercise as an effect of improved aerobic metabolism (19). IH also modifies the number of circulating immune cells, due to the links between ANS, immune system, and hypoxia (20).

In patients with diabetes, abnormal activity of respiratory reflexes (21–26) and reduced responses to hypoxia are frequently observed (27,28), and the immune defense is depressed (29). Altogether, these considerations suggest that IH could induce favorable results in diabetes.

However, as IH has never been applied in patients with type 1 diabetes before, we tested whether a single short bout of IH could elicit favorable changes that improve the hypoxia and the respiratory reflexes and could lead to improved ANS function. In addition, since the responses to hypoxia are multidimensional, we tested its initial effects on the immune system and on the aerobic/anaerobic metabolism at rest by monitoring the lactate production after IH. Finally, we assessed the possible effects of IH on lipid peroxidation and formation of malondialdehyde (MDA), which are markers for cell damage and oxidative stress, respectively, and are generally used when the effects of hypoxia interventions are to be evaluated (30).

The aim of this study was to examine the chain of events occurring after one single bout of IH in patients with type 1 diabetes. For this purpose, we examined cardiorespiratory, metabolic, and hematological responses before and at different times after 1 h of IH and followed the changes over the rest of the same day (6 h).

From the [1]Department of Sport Science, Medical Section, University of Innsbruck, Innsbruck, Austria; the [2]Folkhälsan Institute of Genetics, Folkhälsan Research Center, University of Helsinki, Helsinki, Finland; the [3]Division of Nephrology, Department of Medicine, Helsinki University Central Hospital, Helsinki, Finland; the [4]Department of Internal Medicine, University of Pavia and IRCCS San Matteo, Pavia, Italy; [5]NordLab Oulu, Oulu University Hospital, and Department of Medical Microbiology, University of Oulu, Oulu, Finland; and the [6]IDI Baker Heart & Diabetes Institute, Melbourne, Victoria, Australia.

Corresponding author: Luciano Bernardi, lbern1ps@unipv.it, or Per-Henrik Groop, per-henrik.groop@helsinki.fi.

Received 4 February 2013 and accepted 29 May 2013.
DOI: 10.2337/db13-0167

© 2013 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. See http://creativecommons.org/licenses/by-nc-nd/3.0/ for details.

RESEARCH DESIGN AND METHODS

This placebo-controlled single-blinded study was carried out in fifteen patients with type 1 diabetes (2 females and 13 males) without clinical evidence of respiratory disease or definite autonomic abnormalities. The protocol was approved by the ethics committee of the University of Helsinki, and the study was conducted in accordance with the ethics standards defined in the Declaration of Helsinki. All subjects received extensive information of the study process, and written informed consent was obtained. Inclusion criteria were stable diabetes; absence of infections during the last month; absence of major cardiovascular complications such as coronary heart disease, unstable or stable angina, myocardial infarction, ventricular arrhythmias, and atrial fibrillation; therapy with β-blockers; severe hypertension (>180 mmHg systolic or >110 mmHg diastolic blood pressure); definite autonomic dysfunction; or proliferative retinopathy. Age limit for the selection of subjects was 20–45 years. All patients were on insulin treatment. In addition, five patients received antihypertension medications (angiotensin receptor inhibitors [1] and ACE inhibitors [4]). The patients maintained the same therapy during IH and placebo day. The clinical and anthropometric characteristics of the participants are reported in Table 1.

Measurement sessions were performed on two different days at least 5–7 days apart to exclude possible "learning" effects. On 1 day, the patients underwent the intermittent hypoxic protocol (hypoxia day), whereas on the other day room air was administered using the same protocol (placebo day). Baseline data were obtained in the morning of each testing day at least 2 h after breakfast. Subjects were advised to abstain from caffeinated beverages for 12 h and from alcohol for 36 h prior to testing. For avoidance of possible learning effects from one day to the other, the sequence of hypoxia or placebo day was randomized. Hypoxic or placebo exposures occurred during 1 h in the morning under standardized conditions after completion of baseline (t1) measurements (Fig. 1). During the hypoxia day, each hypoxia session consisted of five hypoxic periods (13% O_2 inspired fraction of oxygen) each lasting 6 min, with five normoxic intervals of same duration. During the normoxia day, the breathing program was performed in the same way, but the subjects inhaled normoxic air. On both days, participants were breathing hypoxic or normoxic air through a facial mask during the 1-h protocol. In each session, blood pressure (Finapres; FMS Medical Systems, Amsterdam, the Netherlands) and heart rate and arterial oxygen saturation (COSMOplus; Novametrix, Wallingford, CT) were continuously measured. In case of a decrease in oxygen saturation <80% or the occurrence of symptoms, hypoxia would have been discontinued until oxygen levels reached at least 80%. A technician regulated and controlled the breathing periods under supervision of a medical doctor in a way that the intervention could not be observed by any patient. Thereafter, three measurement sessions were performed: immediately after (t2), after 3 h (t3), and after 6 h (t4). After t2, each patient obtained an individual meal according to diet requirement that was equal on both testing days. Continuous acquisition of all signals to a personal computer was performed at 600 samples/channel.

TABLE 1
Baseline characteristics of the participants

	Type 1 diabetic patients
Sex (female/male), *n/n*	2/13
Age (years)	36.5 ± 1.2
Height (cm)	180.4 ± 2.5
Weight (kg)	84.4 ± 3.4
BMI (kg/m^2)	25.9 ± 1.1
Waist	92.5 ± 4.3
Hip	99.3 ± 2.2
Waist-to-hip ratio	0.93 ± 0.03
Body fat mass (%)	23 ± 3
Age onset (years)	19.9 ± 1.25
Duration of diabetes (years)	16.7 ± 0.4
Fasting plasma glucose (mmol/L)	9.3 ± 0.9
HbA_{1c} (%)	8.1 ± 0.3
HbA_{1c} (mmol/mol)	65 ± 2
Total cholesterol (mmol/L)	4.4 ± 0.2
HDL cholesterol (mmol/L)	1.5 ± 0.1
LDL cholesterol (mmol/L)	2.8 ± 0.2
Triglycerides (mmol/L)	1.2 ± 0.1
Serum creatinine (μmol/l)	68.3 ± 3.5
Hemoglobin (g/L)	145 ± 2
Autonomic score	0.23 ± 0.12
SBP office (mmHg)	137 ± 3
DBP office (mmHg)	86 ± 2
HR office (bpm)	71 ± 3
Urinary AER (mg/24 h)	2.97 ± 0.39
Smokers, *n*	0

Data are means ± SEM unless otherwise indicated. AER, albumin excretion rate; DBP, diastolic blood pressure; HR, resting heart rate; SBP, systolic blood pressure.

Cardiovascular and respiratory testing. Hypoxic ventilatory response (HVR) and hypercapnic ventilatory response (HCVR) were evaluated to determine respiratory system activity. All patients were tested in the supine position in a silent room at comfortable temperature. Before participants were connected to a rebreathing circuit through a mouthpiece with an antibacterial filter (18,31,32), spontaneous breathing of room air at rest was performed for 4 min in order to obtain baseline data.

During each condition, we performed continuous measurement of oxygen saturation (SaO_2) by a pulse oxymeter and end-tidal CO_2 (CO_2-et) using a capnograph connected to a mouthpiece (COSMOplus). Recordings of electrocardiogram were performed by chest leads, and continuous noninvasive blood pressure was recorded using the cuff method (Finapres). A heated Fleish pneumotachograph (Metabo, Epalinges, Switzerland) connected to a differential pressure transducer (RS part N395-257; RS Components, Corby, U.K.), was inserted in series to the expiratory component of the rebreathing system to measure airway flow.

For measurement of the response to hypoxia, the participants were connected to a rebreathing circuit inducing progressive decrease in SaO_2 while maintaining CO_2-et values at constant levels, until SaO_2 reached 80%, and measuring breath-to-breath changes in minute ventilation. The response to hypercapnia was evaluated by ventilatory changes induced by progressive increase in CO_2-et levels (up to 13 mmHg above resting levels), while SaO_2 was maintained >98% by oxygen at very low flow (18,32).

Cardiovascular autonomic function was determined performing four tests according to recent guidelines (33): deep-breathing, 30:15 ratio, Valsalva maneuver, and systolic blood pressure response to standing. Cardiovascular autonomic neuropathy was defined as the "presence of two or more abnormal tests" (33).

Measurement of chemoreflex sensitivity. The slope of the linear regression line of minute ventilation versus SaO_2 or CO_2-et indicated in each case the chemoreflex sensitivity to hypoxia or hypercapnia. In the hypercapnic test, the point at which the ventilation started to increase was indicated as ventilatory recruitment threshold to CO_2 (VRT- CO_2) (Fig. 2). VRT- CO_2 was identified by interpolating the ventilation/CO_2-et plot by a fourth-order polynomial function (18,32).

Assessment of baroreflex sensitivity. The baroreflex sensitivity (BRS) was measured during spontaneous breathing at each measurement session. Since previous studies did not document a better performance of one method over the others (34), we calculated the average of seven different methods as previously described (35): positive and negative sequences, the α-coefficient in the low- and high-frequency bands and its average, the transfer function technique, and the ratio of SDs of R-R interval and systolic blood pressure variabilities (35). Besides BRS, SD of the R-R interval (SDNN) was applied to determine a global index of heart rate variability. This selection was done based on the fact that normal distribution is more pronounced in this variable compared with other indices of variability (e.g., variance).

Metabolic and hematological evaluation. Venous blood samples were drawn in the morning after a light breakfast and three times after hypoxic/placebo intervention in order to analyze erythrocyte and white blood cell count and for the determination of plasma glucose, HbA_{1c}, hemoglobin content, serum C-reactive protein (sCRP), serum lactate, and creatinine. HbA_{1c} was determined by immunoturbidimetry (Medix Biochemica, Kauniainen, Finland), serum creatinine by routine enzymatic methods, and sCRP by serum immunoprecipitation (ThermoScientific, Vantaa, Finland). One 24-h urinary collection was used to analyze urinary albumin excretion rate by immunoturbidimetry.

Hypoxia-induced lipid peroxidation was measured as the amount of MDA in serum LDL particles using capture sandwich chemiluminescent immunoassay and monoclonal antibody specific for MDA-epitope (clone HMN-08_34) (36). The measurements reported are the ratios of MDA epitopes to total LDL, and the average of two separate assays both carried out using duplicate measurements for each sample was calculated.

Statistical analysis. Data are presented as means ± SEM. SPSS statistical software package 18 was used for data analysis. Probability values ≤0.05 (two-tailed) were considered statistically significant. Unpaired *t* test was used to detect differences between baseline data of both testing days and data obtained from healthy control subjects. In order to assess potential effects

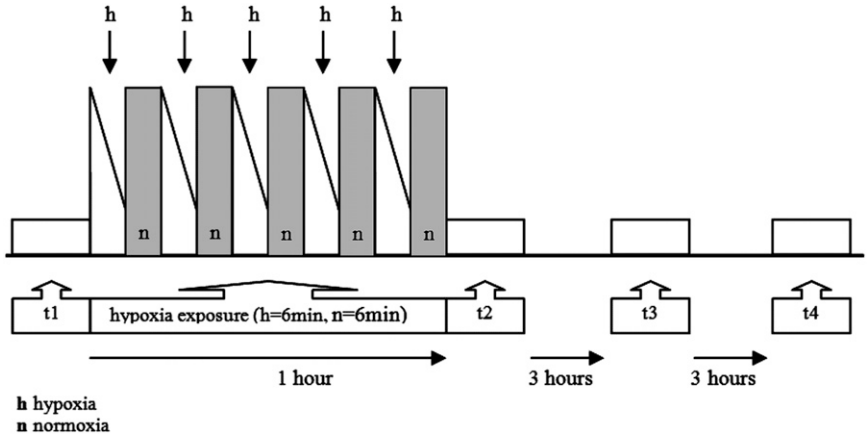

FIG. 1. Diagram of the study protocol, comprising measurements at t1, t2, t3, and t4. The intervention consisted of either *1*) 6-min breathing of 13% oxygen mixture five times, each separated by 6-min recovery (IH) or *2*) placebo exposure (breathing room air with 21% FiO_2 for 1 h) in a single blind protocol. A standardized meal was given to the patients after t2 on both days. IH and placebo days were spaced at least 7 days apart.

generated by the IH, differences in the hypoxia and placebo days versus baseline values were compared using paired *t* test. Moreover, paired *t* test was also used to compare the same time point data between the hypoxia and placebo days and to assess changes from baseline (t1).

RESULTS

All study participants succeeded in performing the hypoxia/placebo protocols and the test sessions. No adverse effects were observed. Complete results of the cardiorespiratory data are summarized in Table 2, and hematological and metabolic variables are presented in Table 3. In the 15 subjects tested, there were 2 subjects with abnormal deep-breathing tests and 1 subject with borderline orthostatic hypotension.

Baseline respiratory and cardiovascular data. Compared with our reference database, baseline HCVR were reduced in the type 1 diabetic patients, and baseline VRT-CO_2 was shifted to the right. Variables obtained at baseline on the hypoxia and the placebo days were not different, verifying the reproducibility of the tests.

Effects of IH

Effects of IH on respiratory data. On the hypoxia day, we observed a significant increase after the intervention in the HCVR (Fig. 3) that persisted until 6 h from the exposure to hypoxia. Even when one subject with large increase in HCVR was excluded from the analysis, the increase was still significant compared with placebo (P = 0.01). A slight increase was seen also on the placebo day, but the extent of the increase was significantly lower than on the hypoxia day. VRT-CO_2 was significantly reduced immediately after the intervention on the hypoxia day, whereas it increased on the placebo day (Fig. 3). HVR also improved after the intervention during the hypoxia day but not during placebo (Fig. 3). This effect still persisted after 3 h from IH, and values tended to be elevated even after 6 h from exposure. Tidal volume, minute ventilation, and respiration rate did not change in the course of the day either during hypoxia or placebo. CO_2-et was significantly elevated after both hypoxia and placebo exposure and persisted until 6 h from exposure on both intervention days. There was no change in oxygen saturation after hypoxia or placebo exposure.

Effects of IH on cardiovascular data. Despite a fairly long exposure to hypoxia, BRS did not show any significant reduction after the intervention. Similarly, no adverse changes were apparent after hypoxia exposure in mean R-R interval or in heart rate variability (SDNN). A transient increase in systolic blood and diastolic blood pressures was observed after intervention (t2) on the hypoxia day; however, blood pressures dropped 3 h later (the change was significant for diastolic blood pressure). During placebo, blood pressures remained unchanged.

Metabolic and hematological data. Plasma glucose concentration decreased after hypoxic and placebo exposure without significant differences between the 2 days (Fig. 4). After 3 h from the intervention, the glucose levels remained reduced on placebo, whereas after hypoxia the values increased toward baseline levels again.

Lactate decreased transiently and significantly after the intervention on the hypoxia day. No such changes were apparent on the placebo day. No changes were observed in erythrocyte counts or hemoglobin concentrations. sCRP remained unchanged. Relative neutrophil count increased 3 and 6 h after IH, whereas relative lymphocyte count diminished 6 h from intervention on the hypoxia day. During

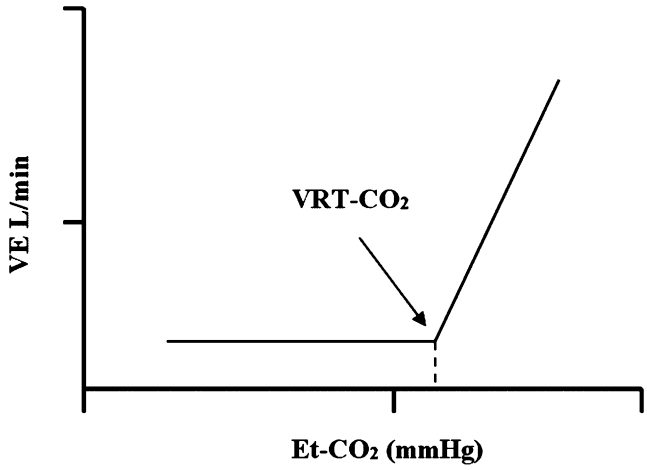

FIG. 2. Outline of the VRT-CO_2 that illustrates the point at which the ventilation started to increase during the progressive HCVR. VRT-CO_2 was identified by interpolating the ventilation/CO_2-et plot using a fourth-order polynomial function. VE L/min, minute ventilation (liters/minute).

TABLE 2
Cardiorespiratory data

	Baseline (t1)	Post (t2)	Post (t3)	Post (t4)
HCVR (L/min^{-1}/mmHg CO_2-et)				
IH day	0.22 ± 0.06	0.39 ± 0.07*#	0.34 ± 0.04#	0.43 ± 0.06#
Placebo day	0.29 ± 0.06	0.26 ± 0.07	0.32 ± 0.06	0.38 ± 0.07
HVR (L/min^{-1}% SaO_2)				
IH day	−0.23 ± 0.04	−0.36 ± 0.07*#	−0.38 ± 0.06*#	−0.35 ± 0.06
Placebo day	−0.43 ± 0.07	−0.30 ± 0.08	−0.26 ± 0.06	−0.40 ± 0.22
VRT-CO_2 (mmHg)				
IH day	44.07 ± 1.19	42.04 ± 0.84**#	45.89 ± 0.91	45.11 ± 0.83
Placebo day	44.14 ± 1.52	44.03 ± 1.12	45.23 ± 1.49#	46.25 ± 0.77#
RR (ms)				
IH day	960 ± 38	980 ± 39	935 ± 41	932 ± 45
Placebo day	1,012 ± 56	1,075 ± 68	960 ± 42	1,004 ± 61
SDNN (ms)				
IH day	41.8 ± 3.64	44.9 ± 3.57	35.6 ± 4.21	42.2 ± 4.98
Placebo day	38.4 ± 5.36	41.8 ± 4.87	37.2 ± 3.73	43.3 ± 6.89
SBP (mmHg)				
IH day	123 ± 4	133 ± 3#	119 ± 3	124 ± 4
Placebo day	121 ± 4	125 ± 4	121 ± 5	124 ± 4
DBP (mmHg)				
IH day	57 ± 2	63 ± 2#	52 ± 3#	58 ± 2
Placebo day	54 ± 2	57 ± 3	55 ± 3	53 ± 4
SaO_2 (%)				
IH day	97.43 ± 0.12	97.47 ± 0.18	97.34 ± 0.16	97.45 ± 0.13
Placebo day	97.38 ± 0.17	97.37 ± 0.21	97.29 ± 0.19	97.29 ± 0.14
BRS (ms/mmHg)				
IH day	11.98 ± 1.36	10.23 ± 1.20	9.13 ± 1.07	9.43 ± 1.18
Placebo day	9.30 ± 1.49	11.09 ± 1.62##	9.84 ± 1.41	9.83 ± 1.15
Respiration rate (breaths/min)				
IH day	11.9 ± 0.9	11.6 ± 0.9	12.8 ± 1.1	12.7 ± 1.2
Placebo day	12.1 ± 0.8	12.0 ± 1.1	12.2 ± 0.9	13.4 ± 1.0
VE (L/min)				
IH day	11.0 ± 1.2	9.9 ± 0.7	10.6 ± 0.7	11.9 ± 1.0
Placebo day	10.6 ± 1.0	10.5 ± 0.9	11.0 ± 0.8	11.1 ± 0.8
Vt (L)				
IH day	956.6 ± 75.4	920.4 ± 99.7	866.3 ± 53.4	1,051.3 ± 159.3
Placebo day	903.5 ± 100.1	1,014.6 ± 169.4	947.6 ± 78.1	879.0 ± 70.3
CO_2-et (mmHg)				
IH day	38.1 ± 1.6	42.1 ± 1.0##	43.2 ± 0.8##	42.3 ± 1.0##
Placebo day	37.5 ± 1.6	41.0 ± 1.0##	42.6 ± 1.2###	42.9 ± 1.1##

Data are means ± SEM. DBP, diastolic blood pressure; SBP, systolic blood pressure; VE, minute ventilation; Vt, tidal volume. *$P < 0.05$ comparing Δ (t − t1) IH vs. Δ (t − t1) placebo days. #$P < 0.05$ compared with t1 of same day. **$P < 0.01$ comparing Δ (t − t1) IH vs. Δ (t − t1) placebo days. ##$P < 0.01$, ###$P < 0.001$, compared with t1 of same day.

the placebo day, the level of MDA in serum LDL declined; however, no changes were observed during IH (Table 3).

DISCUSSION

In the present investigation, we examined the impact of a single bout of IH on the cardiorespiratory function in patients with type 1 diabetes. The major new finding was that IH induces substantial increases in the ventilatory responses to hypoxia and hypercapnia. IH induced also a transient reduction in lactate levels, suggesting improved use of the aerobic pathway. Overall, our findings show potentially useful effects of an approach aimed at improving the adaptation to hypoxia in patients with type 1 diabetes.

As we investigated the effects of IH in type 1 diabetes for the very first time, we excluded subjects with autonomic complications for safety reasons. In addition, because of the unknown effects of IH in these patients, we considered a single bout of IH an intervention adequate for testing "how" the patients would respond to an exposure to IH. In order to test the achievable response, one needs a full IH protocol (lasting several weeks). The observation that no differences were apparent at baseline during the control and hypoxia day verified the reproducibility of our tests. Altogether, based on the positive directional changes in this preliminary study, larger studies of prolonged duration are justified.

Presence of hypoxia in diabetes. Different alterations in diabetes indicate that hypoxia, which may play an important role in the complications of diabetes, is present in type 1 diabetes. In fact, although SaO_2 may remain within normal range, oxygen saturation at rest in diabetic patients is slightly reduced compared with that in healthy control subjects (10). The importance of this finding should not be overlooked: due to the s-shape of the hemoglobin dissociation curve, a small reduction in SaO_2 in the normoxic range implies a much larger reduction in arterial oxygen pressure. Because the arterial oxygen pressure is the actual input to the peripheral chemoreflexes, any reduction in this variable will induce sustained sympathetic

TABLE 3
Hematological data

	Baseline (t1)	Post (t2)	Post (t3)	Post (t4)
Glucose (mmol/L)				
IH day	10.3 ± 0.9	7.4 ± 0.7***	9.7 ± 1.1	10.0 ± 1.1
Placebo day	9.3 ± 0.9	6.8 ± 0.8**	6.1 ± 0.7*	9.1 ± 0.8
sCRP (mg/mL)				
IH day	1.58 ± 0.43	1.60 ± 0.47	1.51 ± 0.45	1.55 ± 0.47
Placebo day	1.8 ± 0.55	1.89 ± 0.62	2.17 ± 0.62	2.24 ± 0.94
Lactate (mmol/L)				
IH day	1.04 ± 0.11	0.88 ± 0.09*	1.01 ± 0.09	1.01 ± 0.12
Placebo day	1.04 ± 0.15	0.98 ± 0.10	1.10 ± 0.15	1.57 ± 0.51
MDA (RU)				
IH day	0.092 ± 0.089	0.089 ± 0.007	0.091 ± 0.008	0.091 ± 0.008
Placebo day	0.113 ± 0.011	0.110 ± 0.009	0.102 ± 0.010*#	0.115 ± 0.012
B-erythrocytes ($\times 10^6/\mu L$)				
IH day	4.70 ± 0.09	4.70 ± 0.09	4.71 ± 0.08	4.70 ± 0.08
Placebo day	4.76 ± 0.10	4.76 ± 0.12	4.80 ± 0.11	4.71 ± 0.10
Hemoglobin (g/L)				
IH day	143 ± 2	143 ± 2	143 ± 2	142 ± 2
Placebo day	144 ± 3	144 ± 3	145 ± 3	143 ± 3
Hematocrit (%)				
IH day	41.2 ± 0.6	40.6 ± 0.6	41.0 ± 0.6	40.4 ± 0.6*
Placebo day	41.5 ± 0.7	41.5 ± 0.8	41.7 ± 0.8	41.1 ± 0.7
Leukocytes (%)				
IH day	52.2 ± 2.7	52.0 ± 2.7	55.7 ± 2.4*	55.9 ± 2.3*
Placebo day	52.4 ± 3.5	52.7 ± 3.1	54.6 ± 3.0	56.0 ± 2.5
Lymphocytes (%)				
IH day	34.3 ± 2.4	34.4 ± 2.0	32.6 ± 2.2	31.9 ± 2.0*
Placebo day	34.0 ± 3.2	33.6 ± 2.1	33.5 ± 2.8	31.9 ± 2.7

Data are means ± SEM. RU, relative unit. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, compared with t1 of same day. #$P < 0.05$ comparing Δ (t 2 t1) IH vs. Δ (t 2 t1) placebo days.

activation. Evidences of hypoxia stem from the evidence of glycosylation of basal membranes in the lungs, leading to lung diffusion abnormalities (reduced diffusing capacity for carbon monoxide) (7). Skin oxygenation ($TcPO_2$) was found to be reduced (though interpreted as insufficient vascular control in several studies) (4), and abnormal blood flow, evidenced by increased venous PO_2 compatible with arterio-venous shunting, was observed (5). The decrease in oxygen transport as a result of glycosylation of hemoglobin shifts the oxygen dissociation curve to the left, thus making O_2 release to the tissue difficult (6). Obstructive sleep apnea (OSAS) (8) is a frequent finding in diabetes. Finally, growing evidence in patients with diabetes indicates impaired responses to hypoxia (27,28).

Effects of intermittent hypoxic exposure. IH, a technique developed for adapting the body to the consequences of hypoxia, has been applied clinically by Russian physicians to elderly people and to patients with various diseases for many years (37,38). We have reported improved exercise tolerance in different diseases (14,19) and improved cardiovascular and respiratory reflexes in patients with chronic obstructive pulmonary disease (22). Similarly, a positive effect of IH on peripheral chemoreflex sensitivity emerged in healthy people (32).

The term "intermittent hypoxia" is often linked to OSAS, leading to elevated sympathetic activity (due to diminished oxygen supply to the organs and tissues) and increased risk for hypertension and cardiovascular diseases (11,39). In contrast, IH can generate beneficial effects (14,19). OSAS is actually characterized by very short exposures to hypoxia (approximately 30 s) that arise many times (hundreds) during nighttime, leading to a stress response because of lack of time for a compensatory effect (40). In contrast, IH is defined by exposures of longer duration (5 min–1 h), with less repetitions per day (1–5) and more time for recovery (>5 min). IH repeated over a few weeks induced an increase in parasympathetic activity and an improvement in BRS (18) (similar to the effects of physical training), whereas OSAS induces sympathetic hyperactivity and reduces BRS (similar to a stress response).

Alterations of respiratory control. Although some investigations did not find alterations in HVR (41) or HCVR (42), many studies indicate that ventilatory responses to hypoxia (21–24) or hypercapnia (23–26) are decreased in diabetic patients, possibly increasing the risk for severe cardiovascular diseases. In the present investigation, the baseline hypercapnic response was subnormal and VRT-CO_2 was higher than normal. As a consequence, ventilation starts to increase only at higher CO_2-levels, and the same respiratory stimulus, i.e., CO_2 levels, result in a lower respiratory activity, respectively.

In this study, IH resulted in an increased activity in both peripheral and central chemoreflexes. Our finding of an evident increase in HCVR initiated by IH concurs with the observation from a previous study evaluating the effects of IH in patients with chronic obstructive pulmonary disease (18), whereas the elevation in HVR is in line with previous findings from a study in healthy subjects, showing an increased HVR after a 2-week intervention of IH (32). In the present investigation, we were able to show that the first changes already appeared after only one single session of IH. These early changes are compatible with an early adaptation (training effect) of the respiratory system.

Interestingly, the increase in HVR was possible by virtue of the decrease in VRT-CO_2 observed after the intervention. If VRT-CO_2 remained elevated, then ventilation

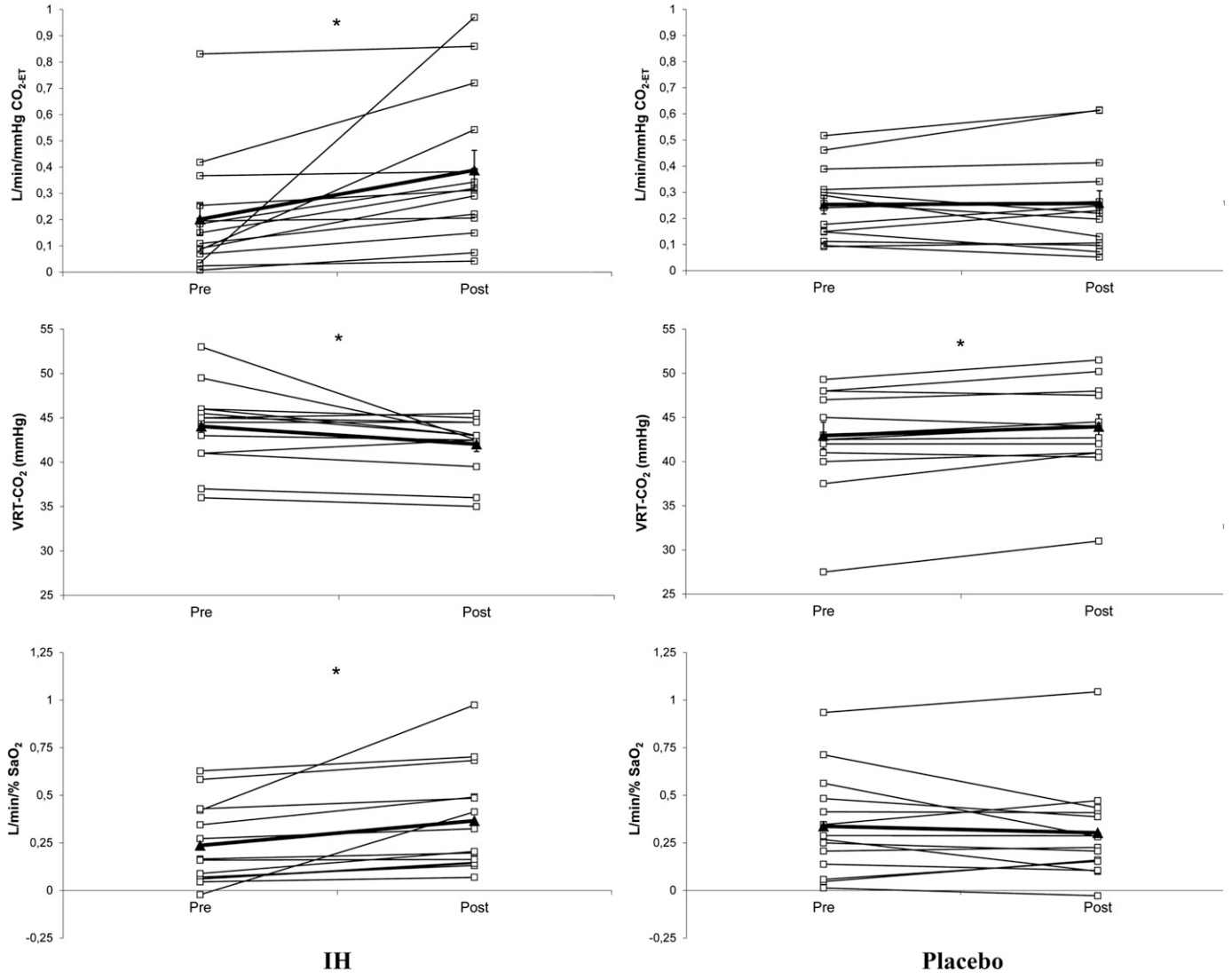

FIG. 3. Plot shows changes in HCVR (*top panel*), VRT-CO_2 (*middle panel*), and HVR (*bottom panel*) immediately after one single hour of IH or placebo exposure (n = 14). *Significant differences ($P < 0.05$, paired t test) from t1 to t2. Thick lines show mean values ± SEM.

could not be sustained during hypoxia, due to the consequent hypocapnia. Therefore, a shifting to the left of the VRT-CO_2 enables an increase in the activity of the peripheral chemoreflex (43). This same phenomenon is normally observed (to a greater extent) during the early phases of acclimatization to hypoxia induced by high altitude. Therefore, our findings seem to imitate an adaptive process normally occurring under exposure to hypoxia.

Alterations of the cardiovascular control. Generally, impaired cardiovascular control (evidenced by blunted BRS or diminished heart rate variability in diabetes [9,44]) is associated with increased morbidity in patients with diabetes (1,2). Previous findings from our group showed a decreased BRS in patients with type 1 diabetes, which nevertheless increased above normal levels by interventions such as deep breathing (3) or short-term oxygen administration (9), indicating that these autonomic impairments might be at least at an initial stage of the disease of a functional origin. Similarly, a full IH protocol improved a blunted BRS in patients with chronic obstructive pulmonary disease (18), although a reduction in BRS is normally seen in hypoxia. After one bout of IH, we did not find a reduction in BRS. We cannot exclude that this could be due to the relatively well-preserved autonomic status of our patients. Alternatively, a potential increase in BRS even after the hypoxia could have been suppressed by the insulin treatment in our participants. Insulin is known to stimulate sympathetic nervous system activity in turn depressing the vagal arm of the BRS (45). We observed an increased systolic and diastolic blood pressure immediately after IH exposure. This might be the result of sympathetic stimulation or a direct initial effect of reoxygenation on the arterial endothelium (9). The fact that we did not show other evidences of sympathetic activation after hypoxia remains compatible with an initial adaptation process and suggests that progression of the intervention might lead to an increased stimulation of the baroreflex and therefore increase the parasympathetic activity, with minor changes or even reduction in blood pressures (18).

Metabolic and hematological effects of intermittent hypoxia. Plasma glucose decreased similarly after intermittent hypoxic and placebo exposure. Contrary to our expectations, we did not find elevations of lactate levels

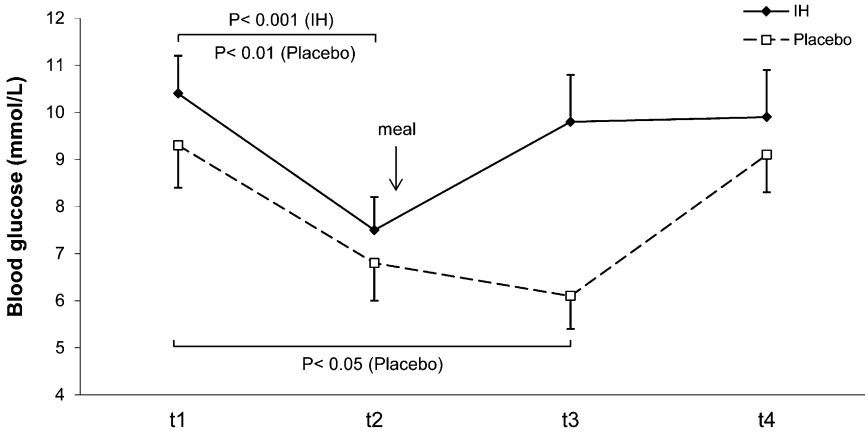

FIG. 4. Blood glucose changes over daytime on hypoxia day and placebo day. Measurements were performed at t1, t2, t3, and t4 after the hypoxic or placebo exposure (n = 15). Standardized meal was taken after t2 on both days (see indication). Blood glucose levels were compared with t1 of the same day. Data are presented as means ± SEM.

after the IH intervention. Generally, acute exposure to hypoxia is known to increase lactate production owing to anaerobic metabolism and also owing to sympathetic activation (46). Conversely, the final effect of a complete IH protocol is a stimulation of the aerobic metabolism that shifts the lactate-load curve to the right during exercise (19). Thus, our results suggest that the aerobic metabolism could have already been stimulated by IH. According to the short duration of the intervention, erythrocyte counts, hemoglobin content, and hematocrit did not show an increase after the intervention. IH increased relative neutrophil count 3 and 6 h after IH and decreased relative lymphocyte count 6 h from intervention on hypoxia day, indicating a first physiological stress response of the body, an effect typically occurring after physical exercise (47) and after acute exposure to hypoxia (20). However, the rise in the ratio of neutrophil and lymphocyte counts (in relative values), a measure of physiological stress (48), did not reach statistical significance over daytime. This might be ascribed again to the very short duration of the intervention performed in our study, and this trend should be confirmed over a full IH protocol. Moreover, sCRP did not change during daytime, suggesting no effect on inflammation. During the placebo day, there was a decrease in the level of MDA in LDL particles over the observation time. Similar reduction was not seen during the intermitted hypoxia. These findings suggest that during IH, there is a moderate increase in lipid peroxidation and formation of free radicals in agreement with previous findings (30). A moderate increase in free radicals was suggested to be an important trigger for a counterregulatory response, similar to what was described for physical training (49). Therefore, if repeated a sufficient number of times, IH might induce physiological stress reactions that potentially might be supportive of an increase of the immune defenses of the body similar to that associated with moderate physical exercise training.

Limitations. In the current study, we selected patients without clinical evidence of autonomic dysfunction, as this was the first time that IH was tested in type 1 diabetes. The responses to the intervention may thus vary in patients with more advanced disease. A marginal exposure to IH took place on the placebo day as well, as the testing of HVR was performed four times a day on the hypoxia and the placebo day. The oxygen applied to test HCVR might have contributed to the blood pressure changes observed in the present investigation.

Conclusion. In conclusion, in type 1 diabetes one single session of IH elicits an initial adaptation in the ANS, showing increased respiratory reflexes. Impaired function of the autonomic system is a common finding in diabetes and might be reversible, at least at an early stage of the disease if originating from a functional impairment. Our findings provide the basis for accomplishing further studies with daily repetitions of IH at a prolonged duration (the IH protocol implemented in our study normally lasts at least 2 weeks) that might strengthen the positive outcome observed with this initial study.

ACKNOWLEDGMENTS

The study was funded by the Academy of Finland, the Folkhälsan Research Foundation, the Wilhelm and Else Stockmann Foundation, the Signe and Ane Gyllenberg Foundation, the Sigrid Juselius Foundation, Medicinska understödsföreningen Liv och Hälsa, the Waldemar von Frenckell Foundation, and a special Finnish governmental grant for health sciences research (no. 7301).

P.-H.G. has received lecture fees from Eli Lilly, Boehringer Ingelheim, Novartis, Genzyme, Merck Sharp & Dohme, Novo Nordisk, and WebMD and is an advisory board member of Boehringer Ingelheim, Novartis, and Cebix. No other potential conflicts of interest relevant to this article were reported.

T.D. drafted the manuscript. L.B. drafted the manuscript, developed the study concept and design, contributed to interpretation and analysis, and edited and revised the manuscript. D.G., A.Sa., A.Sy., C.Fog., J.P.K., H.G., and M.L. researched data and contributed to the discussion. S.H. contributed to data analysis and discussion. C.For. and M.B. edited and revised the manuscript. P.-H.G. supervised the study, contributed to discussion, and edited 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.

Parts of this study were presented in abstract form at the 2012 Meeting of the European Society for the Study of Diabetes, Berlin, Germany, 2–5 October 2012.

The authors acknowledge the skillful technical assistance of Maikki Parkkonen, Jaana Tuomikangas, Tuula Soppela, and Anna-Reetta Salonen from the Folkhälsan Institute of Genetics.

REFERENCES

1. Schönauer M, Thomas A, Morbach S, Niebauer J, Schönauer U, Thiele H. Cardiac autonomic diabetic neuropathy. Diab Vasc Dis Res 2008;5:336–344
2. Bergner DW, Goldberger JJ. Diabetes mellitus and sudden cardiac death: what are the data? Cardiol J 2010;17:117–129
3. Rosengård-Bärlund M, Bernardi L, Fagerudd J, et al.; FinnDiane Study Group. Early autonomic dysfunction in type 1 diabetes: a reversible disorder? Diabetologia 2009;52:1164–1172
4. Boyko EJ, Ahroni JH, Stensel VL. Tissue oxygenation and skin blood flow in the diabetic foot: responses to cutaneous warming. Foot Ankle Int 2001; 22:711–714
5. Boulton AJ, Scarpello JH, Ward JD. Venous oxygenation in the diabetic neuropathic foot: evidence of arteriovenous shunting? Diabetologia 1982; 22:6–8
6. Solomon LR, Cohen K. Erythrocyte O2 transport and metabolism and effects of vitamin B6 therapy in type II diabetes mellitus. Diabetes 1989;38: 881–886
7. Wheatley CM, Baldi JC, Cassuto NA, Foxx-Lupo WT, Snyder EM. Glycemic control influences lung membrane diffusion and oxygen saturation in exercise-trained subjects with type 1 diabetes: alveolar-capillary membrane conductance in type 1 diabetes. Eur J Appl Physiol 2011;111:567–578
8. Choudhury S, Taheri S. Obstructive sleep apnoea and type 2 diabetes: whose disease is it anyway? Practical Diabetes Int 2011;28:183–186
9. Bernardi L, Rosengård-Bärlund M, Sandelin A, Mäkinen VP, Forsblom C, Groop PH; FinnDiane Study Group. Short-term oxygen administration restores blunted baroreflex sensitivity in patients with type 1 diabetes. Diabetologia 2011;54:2164–2173
10. Gordin D, Bernardi L, Rosengård-Bärlund M, et al. Oxygen increases arterial stiffness and blood pressure in patients with type 1 diabetes. Diabetologia 2011;54(Suppl. 1):493
11. Neubauer JA. Invited review: Physiological and pathophysiological responses to intermittent hypoxia. J Appl Physiol 2001;90:1593–1599
12. Casas M, Casas H, Pagés T, et al. Intermittent hypobaric hypoxia induces altitude acclimation and improves the lactate threshold. Aviat Space Environ Med 2000;71:125–130
13. Rodríguez FA, Casas H, Casas M, et al. Intermittent hypobaric hypoxia stimulates erythropoiesis and improves aerobic capacity. Med Sci Sports Exerc 1999;31:264–268
14. Burtscher M, Pachinger O, Ehrenbourg I, et al. Intermittent hypoxia increases exercise tolerance in elderly men with and without coronary artery disease. Int J Cardiol 2004;96:247–254
15. Tin'kov AN, Aksenov VA. Effects of intermittent hypobaric hypoxia on blood lipid concentrations in male coronary heart disease patients. High Alt Med Biol 2002;3:277–282
16. Meerson F, Pozharov V, Minyailenko T. Superresistance against hypoxia after preliminary adaptation to repeated stress. J Appl Physiol 1994;76:1856–1861
17. Meerson FZ, Malyshev IYu, Zamotrinsky AV. Differences in adaptive stabilization of structures in response to stress and hypoxia relate with the accumulation of hsp70 isoforms. Mol Cell Biochem 1992;111:87–95
18. Haider T, Casucci G, Linser T, et al. Interval hypoxic training improves autonomic cardiovascular and respiratory control in patients with mild chronic obstructive pulmonary disease. J Hypertens 2009;27:1648–1654
19. Burtscher M, Haider T, Domej W, et al. Intermittent hypoxia increases exercise tolerance in patients at risk for or with mild COPD. Respir Physiol Neurobiol 2009;165:97–103
20. Thake CD, Mian T, Garnham AW, Mian R. Leukocyte counts and neutrophil activity during 4 h of hypocapnic hypoxia equivalent to 4000 m. Aviat Space Environ Med 2004;75:811–817
21. Nishimura M, Miyamoto K, Suzuki A, et al. Ventilatory and heart rate responses to hypoxia and hypercapnia in patients with diabetes mellitus. Thorax 1989;44:251–257
22. Weisbrod CJ, Eastwood PR, O'Driscoll G, Green DJ. Abnormal ventilatory responses to hypoxia in Type 2 diabetes. Diabet Med 2005;22:563–568
23. Williams JG, Morris AI, Hayter RC, Ogilvie CM. Respiratory responses of diabetics to hypoxia, hypercapnia, and exercise. Thorax 1984;39:529–534
24. Montserrat JM, Cochrane GM, Wolf C, Picado C, Roca J, Agusti Vidal A. Ventilatory control in diabetes mellitus. Eur J Respir Dis 1985;67:112–117
25. Tantucci C, Scionti L, Bottini P, et al. Influence of autonomic neuropathy of different severities on the hypercapnic drive to breathing in diabetic patients. Chest 1997;112:145–153
26. Homma I, Kageyama S, Nagai T, Taniguchi I, Sakai T, Abé M. Chemosensitivity in patients with diabetic neuropathy. Clin Sci (Lond) 1981;61: 599–603
27. Heyman SN, Khamaisi M, Rosen S, Rosenberger C. Renal parenchymal hypoxia, hypoxia response and the progression of chronic kidney disease. Am J Nephrol 2008;28:998–1006
28. Thangarajah H, Yao D, Chang EI, et al. The molecular basis for impaired hypoxia-induced VEGF expression in diabetic tissues. Proc Natl Acad Sci USA 2009;106:13505–13510
29. Geerlings SE, Hoepelman AI. Immune dysfunction in patients with diabetes mellitus (DM). FEMS Immunol Med Microbiol 1999;26:259–265
30. Behn C, Araneda OF, Llanos AJ, Celedón G, González G. Hypoxia-related lipid peroxidation: evidences, implications and approaches. Respir Physiol Neurobiol 2007;158:143–150
31. Bernardi L, Gabutti A, Porta C, Spicuzza L. Slow breathing reduces chemoreflex response to hypoxia and hypercapnia, and increases baroreflex sensitivity. J Hypertens 2001;19:2221–2229
32. Bernardi L, Passino C, Serebrovskaya Z, Serebrovskaya T, Appenzeller O. Respiratory and cardiovascular adaptations to progressive hypoxia; effect of interval hypoxic training. Eur Heart J 2001;22:879–886
33. Spallone V, Bellavere F, Scionti L, et al.; Diabetic Neuropathy Study Group of the Italian Society of Diabetology. Recommendations for the use of cardiovascular tests in diagnosing diabetic autonomic neuropathy. Nutr Metab Cardiovasc Dis 2011;21:69–78
34. Laude D, Elghozi JL, Girard A, et al. Comparison of various techniques used to estimate spontaneous baroreflex sensitivity (the EuroBaVar study). Am J Physiol Regul Integr Comp Physiol 2004;286:R226–R231
35. Bernardi L, De Barbieri G, Rosengård-Bärlund M, Mäkinen VP, Porta C, Groop PH. New method to measure and improve consistency of baroreflex sensitivity values. Clin Auton Res 2010;20:353–361
36. Veneskoski M, Turunen SP, Kummu O, et al. Specific recognition of malondialdehyde and malondialdehyde acetaldehyde adducts on oxidized LDL and apoptotic cells by complement anaphylatoxin C3a. Free Radic Biol Med 2011;51:834–843
37. Meerson FZ, Ustinova EE, Orlova EH. Prevention and elimination of heart arrhythmias by adaptation to intermittent high altitude hypoxia. Clin Cardiol 1987;10:783–789
38. Tkatchouk EN, Gorbatchenkov AA, Kolchinskaya AZ, Ehrenburg I, Kondrykinskaya II. Adaptation to interval hypoxia with the purpose of prophylaxis and treatment. Hypoxia Med J 1994;11:308–328
39. Lévy P, Pépin JL, Arnaud C, et al. Intermittent hypoxia and sleep-disordered breathing: current concepts and perspectives. Eur Respir J 2008;32:1082–1095
40. Lavie L. Obstructive sleep apnoea syndrome—an oxidative stress disorder. Sleep Med Rev 2003;7:35–51
41. Calverley PM, Ewing DJ, Campbell IW, et al. Preservation of the hypoxic drive to breathing in diabetic autonomic neuropathy. Clin Sci (Lond) 1982; 63:17–22
42. Soler NG, Eagleton LE. Autonomic neuropathy and the ventilatory responses of diabetics to progressive hypoxemia and hypercarbia. Diabetes 1982;31:609–614
43. Mahamed S, Cunningham DA, Duffin J. Changes in respiratory control after three hours of isocapnic hypoxia in humans. J Physiol 2003;547:271–281
44. Pikkujämsä SM, Huikuri HV, Airaksinen KE, et al. Heart rate variability and baroreflex sensitivity in hypertensive subjects with and without metabolic features of insulin resistance syndrome. Am J Hypertens 1998;11:523–531
45. Takagi M, Tanaka Y, Yamasaki Y, et al. Responsiveness of insulin-induced cardiac sympathetic nerve activation associates with blood pressure regulation in diabetics. Am J Physiol Endocrinol Metab 2003;284:E1022–E1026
46. Sakata S, Shimizu S, Kishi T, et al. Correlation between erythropoietin and lactate in humans during altitude exposure. Jpn J Physiol 2000;50:285–288
47. Pedersen BK, Hoffman-Goetz L. Exercise and the immune system: regulation, integration, and adaptation. Physiol Rev 2000;80:1055–1081
48. Nieman DC. Exercise, infection, and immunity. Int J Sports Med 1994;15 (Suppl. 3):S131–S141
49. Stapleton PA, Goodwill AG, James ME, Brock RW, Frisbee JC. Hypercholesterolemia and microvascular dysfunction: interventional strategies. J Inflamm (Lond) 2010;7:54