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ORIGINAL ARTICLE

Effects of intermittent hypoxia training on leukocyte pyruvate dehydrogenase kinase 1 (PDK-1) mRNA expression and blood insulin level in prediabetes patients

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Abstract

Purpose Intermittent hypoxia training/treatment (IHT) is an emerging therapeutic approach to alleviate chronic diseases, such as diabetes. The present study investigated the effects of IHT on blood leucocyte pyruvate dehydrogenase kinase 1 (PDK-1) mRNA expression and its relationship with the changes in blood insulin level.

Methods Seven adult healthy volunteers and 11 prediabetic patients participated in this study. A 3-week course of IHT consisted of a 40-min session of 4 cycles of 5-min 12% O_2 and 5-min room air breathing per day, 3 sessions per week for 3 weeks (i.e., total 9 sessions of IHT). Plasma insulin levels and leukocyte PDK-1 mRNA expression were determined at various time points either under fasting condition or following oral glucose tolerance test (OGTT). Correlation between the IHT-induced changes in PDK-1 mRNA and insulin or glucose levels in the same serological samples was analyzed.

Results At pre-IHT baseline, PDK-1 mRNA expression was two times higher in prediabetes than control subjects. IHT resulted in significant augmentation in PDK-1 mRNA expression (> twofold) in prediabetes at the end of 3-week IHT and remained elevated 1 month after IHT, which was correlated with a significantly reduced insulin release and lower blood glucose after glucose loading with OGTT.

Conclusion IHT can trigger beneficial effects in normalizing blood insulin levels in prediabetic patients under oral glucose load, which were closely correlated with an enhanced mRNA expression of PDK-1 in leukocytes. Further clinical trials are warranted to validate the utility of IHT as a non-invasive complementary therapy against diabetes-associated pathologies.

Keywords Hypoxia · Insulin · Diabetes · Pyruvate dehydrogenase kinase · Adaptation · Gene Expression

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Abbreviations

ANOVA	Analysis of variance
GLUT	Glucose transporter
HIF-1α	Hypoxia inducible factor 1α
IHT	Intermittent hypoxia training/treatment
INSR	Insulin receptor
OGTT	Oral glucose tolerance test
PDH	Pyruvate dehydrogenase
PDK	Pyruvate dehydrogenase kinase
PDK-1	Pyruvate dehydrogenase kinase 1
R	Correlation coefficient
SD	Standard Deviation
TCA	Tricarboxylic acid cycle

Introduction

Intermittent hypoxia training/treatment (IHT), which was originated in 1930s in the former Soviet Union for the training of pilots, is now increasingly used both in sports practice and for the prevention and treatment of certain chronic diseases, such as cardiovascular diseases (Mallet et al. 2018; Serebrovskaya and Xi 2016), diabetes (Camacho-Cardenosa et al. 2018; Mackenzie et al. 2012; Morishima et al. 2015; Serebrovskaya 2002), and many others (Navarrete-Opazo and Mitchell 2014). In a recent publication, we described the positive effect of IHT on glucose homeostasis, hypoxia tolerance and some leukocyte mRNA gene expression, i.e., hypoxia inducible factor 1α (HIF-1α), insulin receptor (INSR), facilitated glucose transporter - solute carrier family-2 (SLC2), and potassium voltage-gated channel subfamily J (KCNJ8), in prediabetic patients (Serebrovska et al. 2017). However, the previous study also raised additional questions about how IHT lead to blood glucose reduction as far as 1 month after the end of IHT. Therefore, we decided to use the remaining serological samples collected from the same participants of this small clinical study for further analysis, focusing on the effects of IHT on the pyruvate dehydrogenase kinase 1 (PDK-1) mRNA expression in leukocytes and blood insulin levels in this group of prediabetes patients.

Pyruvate dehydrogenase kinase (PDK) is perhaps the best described mitochondrial metabolic gene target of hypoxia-inducible factor 1 (HIF-1), which controls metabolic flexibility and plays a crucial role in adaptation to hypoxia (Hollinshead and Tennant 2016; Kim et al. 2006). PDK plays a gatekeeper role for the tricarboxylic acid (TCA) cycle controlling quantity of pyruvate feeding into cells via inhibition of pyruvate dehydrogenase complex activity (Nguyen et al. 2016). PDK actively regulates mitochondrial function in hypoxic condition by shunting pyruvate toward lactate, thus permitting continued glycolysis (Huang et al. 2002; Kim et al. 2006; Minchenko et al. 2004; Papandreou et al. 2006). Four known PDK isoforms (PDK-1 to PDK-4) differ in their catalytic activity and responsiveness to the modulators such as NADH and acetyl-CoA, as well as tissue-specific expression (Ferriero et al. 2015). In mammalian species, these isoenzymes of PDK have different binding affinity, phosphorylation site specificity and tissue distribution. Only PDK-1 is capable to phosphorylate all phosphorylation sites, while other isoenzymes can only phosphorylate one site with different rates (Zhou et al. 2016). PDK-1 is present preferentially in the pancreatic islets, heart and skeletal muscles.

PDK-1 is also considered as a potent suppressor of pyruvate dehydrogenase (PDH), especially when blood glucose levels are low and pyruvate can be conserved for gluconeogenesis. Under diabetic conditions, an elevation of PDK gene expression has been implicated in the increased gluconeogenesis in the liver and the decreased glucose utilization in the peripheral tissues (Lee 2014; Peters et al. 2001). Therefore, suppression of PDKs expression (mainly PDK-2 and PDK-4) was considered as a potential mechanism for alleviating the diabetic states (Ferriero et al. 2015; Khan et al. 2017; Kim et al. 2006; Kulkarni et al. 2012).

On the other hand, the transcription of genes involved in glycolysis and its regulation is influenced by insulin. Hypoxia potentiates the glycolytic effect of insulin shifting the balance of the high energy phosphates towards AMP. In addition, this process further limits gluconeogenesis, since the synthesis of glucose is ATP-dependent (Minchenko et al. 2004). It was proved that intermittent hypoxia increases blood insulin levels via inhibition of the islet destruction and promotion of new beta-cell formation in acinar tissue (Kolesnyk et al. 1994, 2013). There is a close relationship between insulin and PDK. In obese and type 2 diabetic animals, both fat and glucose regulate PDK gene and protein expression in islet cells (Xu et al. 2006). Hyperglycemia and hyperlipidemia may contribute to the decline in diabetic islet PDH activity by increasing mRNA and protein expression of PDK. Therefore, the current notion is that, to optimize glucose-stimulated insulin secretion, a low PDK-1 activity has to be maintained to keep PDH in a dephosphorylated and active state (Krus et al. 2010). Interestingly, a more recent study showed that 4-week sustained hypoxia led to decreased blood glucose and increased insulin levels, which were associated with a significantly elevated PDK-2 mRNA expression in mouse liver (Nam et al. 2016).

It was shown in human investigation that acute intermittent exposure to hypoxia decreased insulin sensitivity in healthy adult humans (Peltonen et al. 2012). Other investigators who have used the models simulating obstructive sleep apnea (i.e., very short hypoxic periods under very low oxygen) showed that such mode of intermittent hypoxia leads to impairments in glucose metabolism and causes reductions in insulin sensitivity (Carreras et al. 2012). To the contrary, whereas acute exposure to continuous or short repetitive severe intermittent hypoxia could cause metabolic dysfunction, chronic exposure to moderate intermittent hypoxia may be associated with normalization, or even an enhancement of whole body metabolic function (Lee et al. 2013). To shed light on this intricate question, the present study focused at investigating whether the previously reported beneficial regime of IHT influences HIF-1α–dependent PDK-1 mRNA expression and analyzing possible correlation between PDK-1 expression levels and blood glucose and insulin in prediabetes patients (Serebrovska et al. 2017).

Methods

Characteristics of subjects

Seven healthy volunteers (44–68 years) and 11 prediabetic patients (48–70 years) participated in the current study. The subjects in the healthy control group had no cardiovascular, respiratory, endocrine or central nervous system disorders and their fasting glucose concentration was less than 5.6 mmol/L, and less than 7.8 mmol/L 2 h after a standard glucose tolerance test. Selection of subjects in the prediabetes group was carried out in accordance with recommendations of the expert committee on the diagnosis and classification of diabetes mellitus (Genuth et al. 2003), which include: (1) Impaired fasting blood glucose level (from 5.6 to 6.9 mmol/L); (2) Impaired glucose tolerance, i.e., blood glucose level after 2 h of standard glucose tolerance test (oral intake of 75 g glucose) was from 7.8 to 11.1 mmol/L; and (3) Combined impairments with both elevated fasting blood glucose level and glucose intolerance defined above.

The research protocols, patient health information and informed consent forms were approved by the Ethics Committee of Chebotarev Institute of Gerontology, Kiev, Ukraine. This human study was performed in accordance with the ethical standards according to the 1964 Declaration of Helsinki. All subjects underwent measurements of several anthropometric variables (Table 1), which indicated no significant difference in age and height between *Healthy* and *Prediabetes* groups, whereas the body weight, body mass index, and waist circumference were higher in the prediabetes patients than those of healthy control subjects.

Experiment protocols

All sessions of the present study were conducted in a quiet room at comfortable temperature within a clinical research center of the Chebotarev Institute of Gerontology. Measurement sessions were performed during 2 days before IHT course, 2 days and 1 month after the termination of IHT. For determination of PDK-1 mRNA expression in blood leukocytes, venous blood samples were collected again next day after 1-week IHT course. Patient examination included: (1) anthropometric measurements; (2) determination of PDK-1 mRNA; (3) standard oral glucose tolerance test (OGTT) with plasma insulin determination.

In the morning of the first experiment day, after 3-day routine hospital diet (250–300 g carbohydrates) and normal physical activity, a venous blood sample was drawn under fasting condition from the median antecubital vein for measurement of fasting insulin level as well as genetic analysis. Thereafter, a standard OGTT was conducted according to Ryden et al. (Ryden et al. 2007), which used 75 g glucose mixed in 250 mL water. Venous blood samples were drawn at 120 min after the oral glucose ingestion (2 h post-OGTT). Plasma insulin levels were measured by immunoenzyme method using DRG Insulin ELISA kit (DRG Instruments GmbH, Germany). We also reused the individual blood glucose data from the same subjects reported in our previous article (Serebrovska et al. 2017) for further analyzing if there is any correlation between the changes in blood glucose and leukocyte PDK-1 mRNA expression.

From the next morning, after a light breakfast, all participated subjects received total of nine sessions of IHT (i.e., three times a week for the subsequent 3 weeks). Each session consisted of four cycles of 5-min hypoxia (12% inspired O_2) followed by 5-min normoxia (room air breathing). The normobaric hypoxia was administered to the subjects in sitting position, using a hypoxic apparatus—Hypotron® (Kiev Polytechnic Institute, National Technical University of Ukraine). Next day and 1 month after the end of 3-week IHT, the post-IHT examinations were conducted in the same manner as the pre-IHT ones.

In addition, blood lactate level was determined at two time-points (i.e., Pre-IHT baseline and after the 3-week sessions of IHT) using a SUPER GL lactate analyzer (Dr. Müller Gerätebau GmbH, Freital, Germany).

Measurement of gene expression

mRNA expression of PDK-1 was determined in circulating blood leukocytes collected in various time points using

Table 1 Anthropometric characteristics of the participants

Groups	Gender (female/male)	Age (year)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Waist (cm)
Healthy	5/2	58.7 ± 11.8	170 ± 15	76.1 ± 17.3	27.2 ± 6.4	93.7 ± 9.2
Prediabete	7/4	66.4 ± 5.2	167 ± 10	90.2 ± 9.9	33.2 ± 5.6	99.7 ± 8.9
Healthy versus prediabetes		NS	NS	$p < 0.05$	$p < 0.05$	$p = 0.05$

Data are Mean ± Standard Deviation (SD); Student *t* test was used to determine the statistical significance of the differences between *Healthy* and *Prediabetes* groups

BMI body mass index, *Waist* waist measurements, *NS* no significant difference

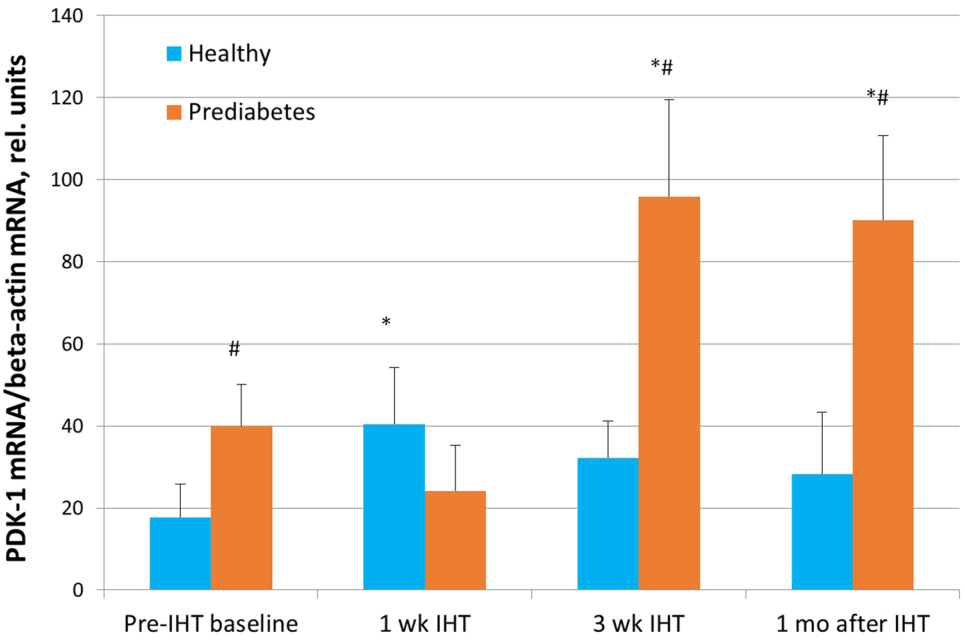

Fig. 1 Time-dependent effects of intermittent hypoxia training (IHT) on pyruvate dehydrogenase kinase 1 (PDK-1) mRNA expression in blood leukocytes. Data are Mean ± SD and were analyzed with two-way ANOVA with repeated measures. Symbols indicate: *$p < 0.05$ versus Pre-IHT baseline; #$p < 0.05$ versus *Prediabetes* group. Group Main Effect (*Healthy* versus *Prediabetes*): $F = 7.185$; $p = 0.017$. Time Effect (3 time-points): $F = 5.837$; $p = 0.029$. Group + Time Effect: $F = 3.822$; $p = 0.068$

real-time polymerase chain reaction (RT-PCR) assay. Blood leukocytes were obtained by centrifuging the blood samples at 1500*g* for 1.5 min. After centrifugation, supernatant with interphase fraction was collected and transferred in new tube. After a secondary centrifugation (3000*g* for 3 min) the supernatant was removed, the precipitate was used for RNA isolation using phenol–chloroform extraction after homogenization with guanidine isothiocyanate (Trizol RNA Prep 100 Kit, Russian Federation). Total RNA concentration was determined with a spectrophotometer (Model ND1000, NanoDrop Technologies Inc., USA). cDNA was synthesized from 5 µg of total RNA by reverse transcription with 10 mM Tris–HCl (pH 9.0), 5 mM $MgCl_2$; 1 mM dNTPs; 20 U RiboLock, Random hexamer primers (0.5 µg $µl^{-1}$) and 200 U RevertAid H Minus M-MuLV Reverse Transcriptase. PCR was performed using an Applied Biosystems 2700 (PerkinElmer, USA).

Gene expression of PDK-1 (Hs00176853_m1) was determined using TaqMan® Gene Expression Assay (Applied Biosystems, USA). The pairs of forward and reverse primers for PDK-1 and the TaqMan® probes for the target mRNA were designed by Applied Biosystems based on the human mRNA sequence. Gene expression in each probe was normalized with β-actin, using a TaqMan® human β-actin control reagent. The thermal cycles of PCR amplification consisted of initial denaturation step at 95 °C for 20 s, followed by treatment at 95 °C for 3 s, and at 60 °C for 30 s and for 50 cycles using 7500 Fast Real-time PCR equipment (Applied Biosystems, USA). The cycle threshold is defined as the number of cycles required for the fluorescence signal to exceed the detection threshold. The expression level of target gene was calculated relative to the housekeeping gene (β-actin) as the difference between the threshold values of the two genes. Each PCR step was performed in duplicate and the calculations were done using the 7500 Fast System SDS software (Applied Biosystems, USA).

Statistical analyses

All data were analyzed using SPSS software version 21.0 (SPSS Inc., USA). Data are expressed as Mean ± Standard Deviation (SD). Statistical significance of the differences between the means of the variables at different time points of IHT was calculated by two-way analysis of variance (ANOVA) with repeated measures. Student *t* test was used for comparing anthropometric characteristics of the participants between *Healthy* and *Prediabetes* groups. Pearson product-moment correlation coefficient (*R*) was calculated to show the degree of linear relationship between blood insulin or glucose and PDK-1 mRNA expression in the fasting and 2 h post-OGTT conditions. The level of statistical significance was set at $p < 0.05$.

Results

In general, all subjects well tolerated the entire process of medical examination and IHT sessions. No subjective discomforts and/or any other adverse effects were reported.

PDK-1 mRNA expression in leukocytes

Initial level of PDK-1 mRNA expression was in two times higher in *Prediabetes* group comparable to *Healthy*

subjects (Fig. 1, $p < 0.05$). During the first week of training it increased in two times in *Healthy* group with a gradual return to the baseline by the end of the training period. In *Prediabetes* group, this increase occurred with a delay until the end of IHT, where PDK-1 mRNA expression increased > twofolds and remained at an elevated level (145%) 1 month after the end of training.

Blood insulin and lactate levels

Figure 2 demonstrates the effects of IHT on blood insulin level in *Healthy* subjects and *Prediabetes* patients. At pre-IHT baseline, fasting insulin in prediabetes patients did not differ significantly from healthy subjects, but 2 h post-OGTT showed triple excess in patients in comparison with healthy volunteers ($p < 0.01$). One day after IHT termination fasting insulin increased by 86% in Healthy group ($p < 0.05$). In Prediabetes patients only the tendency to increase was registered because of the large interindividual dispersion.

One month after IHT the fasting insulin remained at around 66% higher level in *Healthy* group, whereas it returned to the initial level in *Prediabetes* group. Meanwhile, 2 h post-OGTT insulin returned to the baseline in both groups, while the difference in the insulin level under glucose load between the groups remained 2.6 times higher in *Prediabetes* patients ($p < 0.05$). Two-way ANOVA test has shown the statistical difference for fasting insulin in time effect only ($p < 0.01$) and for 2 h post-OGTT insulin level in group main effect ($p < 0.05$), wherein in the group + time effect the difference was close to statistical significance ($p = 0.07$).

Figure 3 provides the evidence that blood lactate concentration was not significantly different between *Healthy* and *Prediabetes* groups either at the pre-IHT baseline or immediately after the end of 3-week IHT, indicating the long-term 9 sessions of IHT (40 min each session) did not activate the cellular signaling cascades leading to enhanced production of lactate via anaerobic glycolysis.

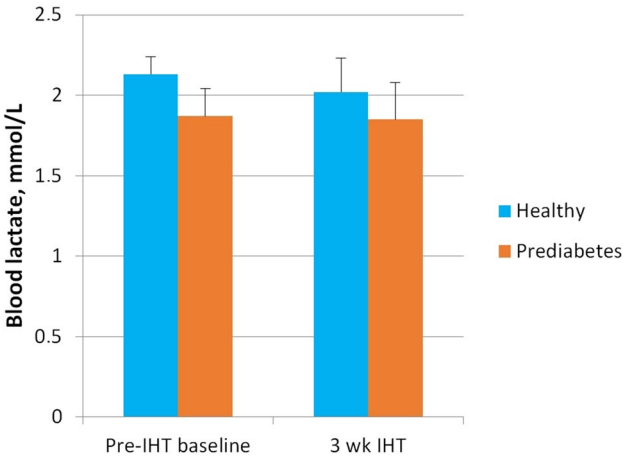

Fig. 3 Blood lactate levels before and after 3-week sessions of intermittent hypoxia training (IHT). Data are Mean ± SD. Student *t* test indicates no significant difference in blood lactate between the end of 3-week IHT versus Pre-IHT baseline as well as between *Healthy* and *Prediabetes* groups at either of these time-points

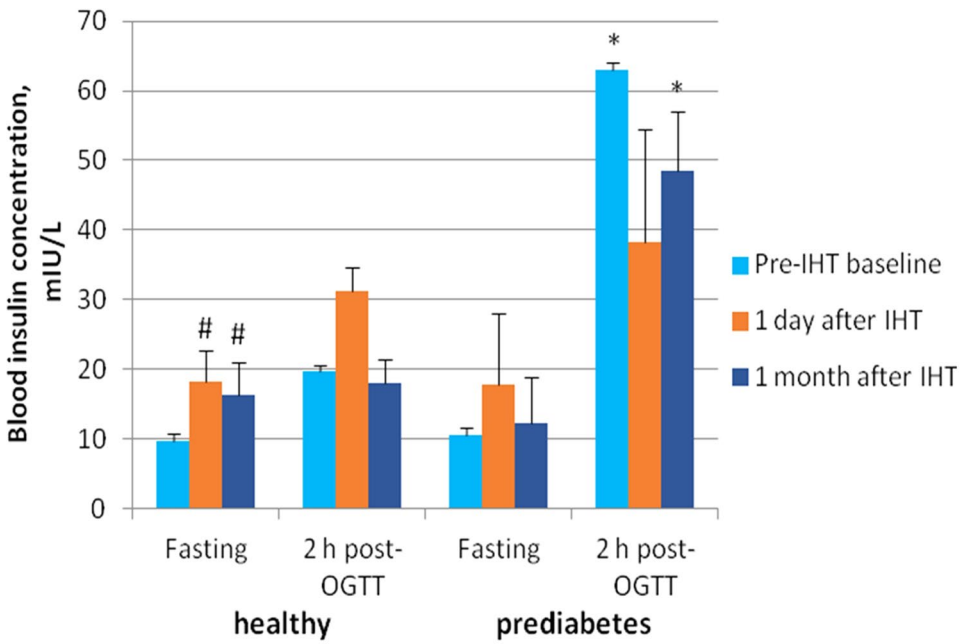

Fig. 2 Blood insulin levels following oral glucose tolerance test (OGTT) at pre-IHT time-point and right after the 3-week sessions of IHT. Data are Mean ± SD and were analyzed with two-way ANOVA with repeated measures. Symbols indicate: *$p < 0.05$ versus Pre-IHT baseline; #$p < 0.05$ versus *Healthy* group

Correlation analysis

Investigation of the links between PDK-1 and other indices showed that the 1 day after IHT, mRNA expression of PDK-1 (the point of the greatest increase in *Prediabetes* group) is positively associated with fasting insulin in this point for all participants (*Prediabetes* group $r = 0.55$, $p < 0.01$; *Healthy group* $r = 0.53$; $p = 0.05$; Fig. 4a) and negatively—with the level of 2 h post-OGTT glucose (*Prediabetes* group $r = -0.47$, $p < 0.05$; *Healthy group* $r = -0.46$; $p =$ NS; Fig. 4b). It means that the subjects with higher PDK-1 expression after hypoxic training had higher level of fasting insulin and lower level of blood glucose after glucose load. This fact is confirmed by the data in Fig. 4c, i.e., the higher the level of PDK-1 to the end of training course, the more the level of 2 h post-OGTT glucose decreases in comparison with the basal point (*Prediabetes* group $r = -0.55$; $p < 0.01$; *Healthy group* $r = -0.59$, $p < 0.05$).

Figure 5 shows the relationships between changes in PDK-1 mRNA expression in human leukocytes 1 month after the termination of 3-week IHT and blood insulin levels under glucose load at 2-h post-OGTT time-point. We observed that in the prediabetic patients, the increased expression in PDK-1 mRNA was positively correlated with more glucose-stimulated insulin secretion at baseline point (Fig. 5a, $r = 0.66$, $p < 0.01$). Conversely, at 1 month after

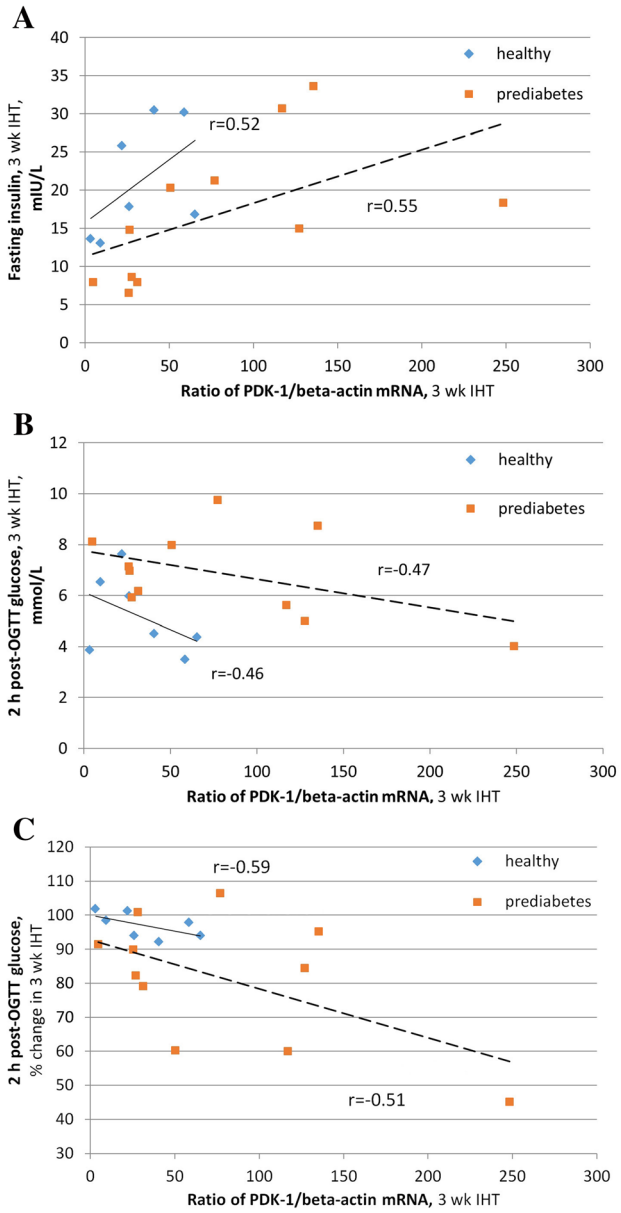

Fig. 4 Relationships between pyruvate dehydrogenase kinase 1 (PDK-1) mRNA expression in human leukocytes after 3-week sessions of intermittent hypoxia training (IHT) and other parameters. **a** PDK-1 mRNA expression and fasting blood insulin levels. **b** PDK-1 mRNA expression and 2 h post-OGTT glucose levels. **c** PDK-1 mRNA expression and % changes in 2 h post-OGTT glucose levels between the pre-IHT baseline and after 3-week IHT

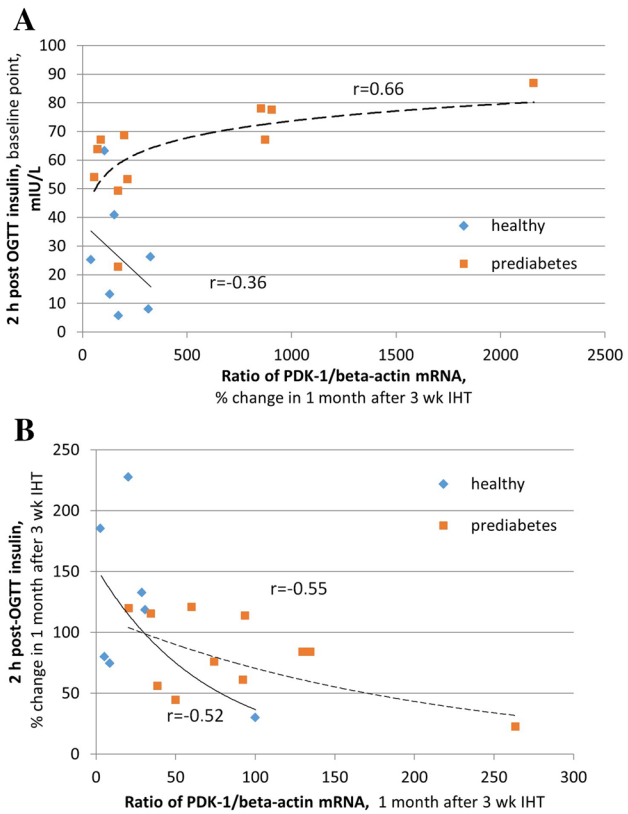

Fig. 5 Relationships between changes in pyruvate dehydrogenase kinase 1 (PDK-1) mRNA expression in human leukocytes 1 month after IHT termination and blood insulin levels at baseline and under glucose load. **a** Correlation between the % changes in leukocyte PDK-1 mRNA expression 1 month after 3-week IHT and blood insulin levels at baseline time-point and 2 h post-OGTT. **b** Correlation between leukocytes PDK-1 mRNA expression 1 month after 3-week IHT and % changes of 2 h post-OGTT blood insulin levels at 1 month after 3-week IHT

IHT time-point, the prediabetes patients with higher PDK-1 mRNA expression had lower insulin release in response to glucose load (Fig. 5b, $r = -0.55$, $p < 0.05$), suggesting PDK-1 inhibits insulin secretion in beta cells.

Discussion

The present study is an expanded analysis for a new target in the same serological samples obtained from the same groups of subjects included in our recent publication (Serebrovska et al. 2017), in which we observed that 3-week IHT reduced fasting and 2-h post-OGTT blood glucose in prediabetes patients, significantly increased their tolerance to acute hypoxia, and upregulated HIF-1α mRNA expression as well as several HIF-1α-regulated genes in blood leukocytes. The most salient finding of the current study is the identification of the involvement of PDK-1, which plays an important role in regulation of post-OGTT blood insulin and glucose levels, the good indicators of glucose tolerance.

The beneficial effects of IHT observed in our present study in prediabetes appear to be conceptually in agreement with other recently published works with various modes of IHT in normal sedentary subjects (Morishima et al. 2015) or elderly cardiac patients with comorbidities (Dudnik et al. 2018). Concerning the possible mechanistic explanations of the benefits of IHT, it was previously reported that acute severe hypoxia, as well as hyperglycemia, induced HIF-1α and PDK-1 protein in pancreas cells of experimental animals and modified glucose metabolism (Nguyen et al. 2016). Another mouse study also showed that 4-week continuous hypoxia resulted in decreased blood glucose level and increased insulin levels, along with a significantly elevated PDK-2 mRNA expression in liver (Nam et al. 2016).

It is noteworthy that our results on the IHT-induced increase in PDK-1 mRNA expression and alleviation of hyperglycemia along with improved insulin sensitivity in prediabetic patients appear to be contradictory with several studies suggesting increased PDK activity would lead to a deterioration in metabolic disorders. These authors suggested that PDK family members phosphorylate PDH complex preventing the incorporation of pyruvate into oxidative phosphorylation process, and in turn leading to elevated anaerobic glycolysis and decreased cellular respiration (Kim et al. 2006; Sutendra and Michelakis 2013; Park et al. 2018; Wu et al. 2018). Inactivation of PDH results in pyruvate conversion to lactate. This glycolytic metabolic shift has been outlined in diverse pathological conditions, including diabetes. It was assumed that the inhibition of PDKs could be a beneficial approach in treating metabolic diseases (Jeoung 2015; Wu et al. 2018). Inhibition of PDKs augmented usage of the glycolysis-produced pyruvate in the mitochondria and in turn increased oxidative phosphorylation (Khan et al. 2017).

To the contrary, a favorable role of upregulated PDK under hyperglycemic conditions was also suggested (Sugden et al. 2001), in which PDK-1 expression may be important for the intensified utilization of glucose and lipids by pancreatic islets. PDK activity may be important under hypoxic conditions, when the energy metabolism mainly shifts to the use of lipid substrates (Portnichenko et al. 2012b). The importance of PDK-1 functionality was also supported by the fact that PDK-1 inhibitors could have negative effects on hemopoiesis and skeletal muscle function (Halvarsson et al. 2017; Nguyen et al. 2016). Nevertheless, the function of PDK-1 isoform has been much less understood either in the development of diabetes, or in adaptation to hypoxia. Previous studies mostly focused on PDK-2 and PDK-4, which were elevated in patients with type 2 diabetes and the exact molecular mechanisms remain unclear (Kim et al. 2006; Kulkarni et al. 2012). Our present investigation indicated that mRNA expression of PDK-1 in patients with prediabetes was > twofold higher than healthy controls (Fig. 1). Following IHT, PDK-1 gene expression in healthy subjects increased at the 3rd session of hypoxic exposure and subsequently returned to the baseline, which is paralleled with the change pattern of HIF-1α described in our recent publication (Serebrovska et al. 2017). On the other hand, PDK-1 in prediabetic patients had a delayed and more persistent elevation up to 1-month post-IHT. Again, this change in PDK-1 was in accordance with the previously described the pattern of changes in other HIF-1α target genes, namely insulin receptor (INSR) and potassium voltage-gated channel subfamily J (KCNJ8) (Serebrovska et al. 2017). Other studies in animals reported that intermittent hypoxia coincidentally increased protein levels of PDK-1 and HIF-1 in skeletal muscle (Nguyen et al. 2016). In addition, Costalat et al. recently showed that single IHT session altered the intensity of glycolysis, increased blood lactate and decreased glucose level in health men (Costalat et al. 2018). The post-IHT hypoglycemic effect (Serebrovska et al. 2017) might be attributable to increased glycolytic processes, the so-called Pasteur effect (Sakata et al. 2000). However, we found no changes in blood lactate levels in the present study (Fig. 3), which refutes such an explanation. Nevertheless, a short-term activated glycolysis in various tissues during the brief hypoxic episodes of IHT may not be completely ruled out, but this process cannot be extended to the subsequent steady-state normoxic condition when we measured the blood lactate samples under air breathing at the next day after 3-week course of IHT.

Regarding the changes in blood insulin under hypoxic conditions, there is a wide range of contradictory opinions. An increased synthesis of insulin in the pancreas at moderate high altitudes has been considered as a possible mechanism for the development of hypoglycemia in

non-adapted organisms (Essop 2007; Roberts et al. 1996). During long-term adaptation to hypoxia, an enhanced glucose metabolism is likely provided through the induction of glucose transporter 1 (GLUT-1) and stress-reactive regulation (Portnichenko et al. 2012a). Most recent report showed that long-term hypoxic exposures of similar magnitude and duration, but consisting of different patterns (i.e., sustained hypoxia versus intermittent hypoxia) elicited discrepant effects on visceral white adipose tissues insulin sensitivity in mice, which may reflect different trajectories of HIF-1a transcriptional activity (Gozal et al. 2017). On the other hand, in response to short-term hypoxia, a decrease in plasma glucose response for glucose loading in healthy people was reported, likely due to a shift in the hormonal milieu that increased glucose utilization, not insulin per se (Hao et al. 2015; Kelly et al. 2010). Mackenzie et al. investigated the effect of single 60-min hypoxic exposure (~ 14.7% O_2) in combination with exercise in the patients with type 2 diabetes and showed improvement in fasting insulin resistance index at 24- and 48-h time points after the hypoxic exposure. In addition, Tian et al. investigated type-2 diabetic rats underwent the adaptation to simulated altitude of 5000 m (6 h per day for 28 days) (Tian et al. 2016) and they observed significant anti-diabetes effects by this type of IHT through ameliorating insulin resistance via hepatic HIF-insulin signaling pathway. Another study compared the effects of different duration of IHT (2 weeks versus 4 weeks) on glucose metabolism (Morishima et al. 2015) and the authors found the area under the curve for serum insulin concentrations after glucose ingestion significantly decreased after 4-week IHT suggesting a longer period of IHT afforded greater improvement in insulin sensitivity.

However, prolonged exposures to severe intermittent hypoxia (from 12.5 to 5% O_2, 8 h/day for 12 weeks) reduced the insulin/proinsulin ratio in the pancreatic tissue, and caused pancreatic tissue lesions and cells apoptosis in a hypoxia dose-dependent manner (Wang et al. 2017). In healthy human volunteers, treatment with 5-h intermittent hypoxia simulating obstructive sleep apnea decreased insulin sensitivity and glucose effectiveness (Louis and Punjabi 2009). However, another human study with 3-h exposure to severe intermittent hypoxia, simulating sleep apnea (25-s exposures to 5% O_2 followed by 2-min normoxia) failed to find any change in insulin sensitivity using OGTT (Newhouse et al. 2017).

It is also noteworthy that hypoxia-enhanced glucose transport via cellular pathways independent of insulin often produces a false impression of insulin resistance (Mackenzie and Watt 2016), since hypoxia may decrease insulin signaling but may not induce whole body insulin resistance. Our present study did not observe significant changes in fasting blood insulin following 3-week IHT, but insulin release in response to glucose loading significantly decreased (Fig. 2). Moreover, the subjects with higher level of PDK-1 expression after IHT had lower post-OGTT blood glucose concentration (Fig. 4c). These data suggested a possible role of PDK-1 as a negative regulator in regulating glucose-stimulated insulin secretion by pancreatic beta cells in patients with prediabetes. The prolonged expression of PDK-1 may gradually achieve normalization of insulin secretion in response to glucose load. Conversely, induction of PDK-1 in healthy individuals was not maintained 1 month after the termination of IHT, since they had no defective glucose-stimulated insulin secretion. Thus, the hypoxic induction of PDK-1 and possibly other PDK isoform(s) may play an important role in regulating not only the cellular processes of glucose utilization, but also the insulin-dependent and insulin-independent glucose transport into cells.

Based on these initial and limited pieces of evidence, we postulate that in patients with prediabetes, there are disturbed insulin-dependent regulation of glucose and lipid metabolism and substrate deficiency for synthesis of high-energy phosphates. Such disorders would modify the cellular metabolism with the possible transition to glycolysis or the preferential use of lipid substrates. Assuming the induction of PDK-1 by IHT is found most pronounced in muscle tissues that ensure the possible emergency transition to the glycolytic pathway of ATP synthesis, if severe hypoxia has developed in the tissues. As we previously reported, even under moderate hypoxia, a switch to more efficient use of lipid substrates for cells may take place (Portnichenko et al. 2012b). The initial metabolic change may include enhanced glucose utilization via induction of insulin-dependent glucose transporter 4 (GLUT-4) and insulin-like growth factor 1 (IGF-1), whereas during prolonged exposure to hypoxia, insulin-independent glucose transporter 1 (GLUT-1) may also be upregulated (Portnichenko et al. 2009), which regulates energy metabolism even under the conditions of impaired insulin sensitivity and/or insulin synthesis. Reduction of glucose-stimulated insulin response observed after IHT may be mediated by PDK-1 induction in pancreatic cells and its negative regulatory action on insulin secretion under hyperglycemia.

Conclusion

Our present study provided novel evidence showing 3-week IHT can trigger beneficial effects in lowering post-OGTT blood insulin level in prediabetes patients, indicating an improved insulin sensitivity. In their leucocytes, IHT-induced activation of HIF-1 led to further increase in PDK-1, a HIF-1 target gene that may be responsible for glucose metabolism with a long-lasting manner after the termination of IHT. The increased PDK-1 following IHT was insufficient for marked glycolysis activation and significant lactate

production and accumulation. Further studies are needed to validate the current findings obtained from the small number of patients in a single center and it is also warranted to determine the most effective mode and dose of hypoxia for an ultimate use of IHT as a non-invasive, complementary preventive and/or therapeutic approach against diabetes.

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Author contributions All authors participated in the design and interpretation of the studies, data analysis, review, and final approval of the manuscript. TVS designed the study and wrote the manuscript. TVS, AGP, VIP, EE, IAS, SN, and VBS elaborated the study protocols and performed statistical analyses of the results. LX critically edited and final-assembled the manuscript. VBS provided the enrollment and clinical examination of the subjects as well as general research management.

Compliance with ethical standards

Conflict of interest LX is a co-founder of Xiamen Innovo Medical Technology Co. Ltd., Xiamen, China and EE is an owner of CellGym Technologies GmbH, Berlin, Germany. All other authors declare no potential conflicts of interest with respect to the research, authorship, and publication of this article.

Ethical approval The research protocols, patient health information and informed consent forms were approved by the Ethics Committee of Chebotarev Institute of Gerontology, Kiev, Ukraine. This human study was performed in accordance with the ethical standards according to the 1964 Declaration of Helsinki.

Informed consent Informed consent was obtained from all individual participants included in this study.

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