Acute Hypoxic Test in Patients with Prediabetes

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ACUTE HYPOXIC TEST IN PATIENTS WITH PREDIABETES

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Abstract

Prediabetes is a state of impaired carbohydrate metabolism when not all of the symptoms required to label a person as diabetic are present, but blood glucose is higher than in healthy subjects. Recent evidence suggests that intermittent hypoxic training (IHT) might provide a cost effective strategy for improving metabolic functioning. One of the most important aspects of the successful IHT application is individualized approach to hypoxic dose and regimen prescription. To establish the relationships between indices of carbohydrate metabolism and individual resistance to hypoxia, the acute hypoxic test (AHT, breathing gas mixture with 12 % O_2 during 20 min) was performed in 33 healthy volunteers (mean age, 63,0, range, 44–76; fasting plasma glucose less than 5.6 mmol/L and 2 h post-OGTT glycemia less than 7.8 mmol/L) and 30 patients with impaired glucose metabolism (mean age, 65,5, range, 44–75; fasting plasma glucose from 5.6 to 6,9 mmol/L and 2 h post-OGTT glycemia from 7.8 to 11 mmol / L). Negative correlation was found between the SaO_2 level at 20^{th} min AHT and fasting plasma glucose ($r = -0,83$; $p<0,01$) and insulin ($r = -0,27$; $p<0,05$) as well as 2 h post-OGTT glucose and insulin levels ($r = -0,75$ and $-0,40$, respectively). Longer recovery time, less effective functioning of respiratory and cardiovascular systems was also registered in patients with prediabetes showing that their cardiovascular resilience is impaired compared to normoglycaemic controls. These patterns of relationship must be considered when assigning the individual modes of IHT.

Key words: prediabetes, acute hypoxia test, glucose, insulin, oxygen blood saturation.

Introduction

Metabolic disorders represent one of the major health and economic burdens for modern society. Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Type 2 diabetes (previously referred to as non-insulin-dependent diabetes) encompasses individuals who have insulin resistance and usually have relative (rather than absolute) insulin deficiency (American Diabetes Association, 2004). Prediabetes is a state of impaired carbohydrate metabolism when not all of the symptoms required to label a person as diabetic are present, but blood sugar is higher than in healthy subjects. This stage is often referred to as the "grey area". According to American DiabetesAssociation (ADA) diagnostic criteria (Ryden et al, 2013), prediabetic metabolic disorders are characterized by impaired fasting glucose (IFG) when fasting plasma glucose (FPG) levels are from 5.6 mmol/l (100 mg/dl) to 6.9 mmol/l (125 mg/dl), impaired glucose tolerance (IGT) when 2-h values in the oral glucose tolerance test (OGTT) are from 7.8 mmol/l (140 mg/dl) to 11.0 mmol/l (199 mg/dl), and their combination. For today about 314 million subjects with this pathology are registered in the world and their number is prognosticated to increase up to 500 million in 20 years.

Recent evidence suggests that hypoxic exposures might provide a cost effective strategy for improving metabolic functioning. More than 20 years ago Ukrainian scientists have proved that intermittent hypoxic exposures reduce risk factors in diabetic animals, increase blood insulin level, inhibit the islet destruction, provide new formation of beta-cells in acinar tissue, and decrease the glucagon and somatostatine production (Kolesnyk et al., 1994). Later on, Prysiazhna et al. (2007) reported a restoring effect of intermittent hypoxia training (IHT) on endothelial function in experimental diabetes mellitus. Recently, these results were confirmed and specified by other authors (Chen et al., 2010; Tekin et al., 2012; Kolesnik et al., 2012). IHT glucose-lowering effect was also confirmed on patients with

diabetes mellitus (Kolchinskaya et al., 1999; Urdampilleta et al., 2012; Morishima et al., 2014). Now there is no doubt that IHT increases the activity of glycolytic enzymes, enhancing the number of mitochondria and glucose transporter levels as well as improving insulin sensitivity.

One of the most important aspects of the successful application of IHT methods into medical practice is individualized approach to the prescription of dose and regimen of hypoxic exposure. Although all individuals respond to the drop in arterial oxygen saturation (SaO_2), the pattern and magnitude of the response as well as the hypoxia resistance per se significantly varies from person to person (Berezovskii and Serebrovskaia, 1988; Serebrovskaya and Xi, 2012). In order to establish an individual's type of hypoxia reaction it is advisable to complete a hypoxic test for each person before they start a course of IHT. The acute hypoxic test (AHT) consists of short term (about 20 min) breathing of hypoxic air of known oxygen concentration followed by a recovery period, when the person takes off the mask and reverts to normal (ambient) air breathing. The purpose of this test is: (a) to determine the functional state of a patient; (b) to establish the type of individual reaction to hypoxic air breathing; (c) to prescribe the most efficacious treatment for an individual in order to cater for individual hypoxic tolerance and current functional state (Korkushko et al, 2005; Bassovitch and Serebrovskaya, 2009).

This fact is particularly important in the elderly - the age of greatest risk in developing diabetes. There is the evidence that in older people the tolerance to lack of oxygen decreases against the background of morphological and functional changes that accompany aging (Korkushko et al., 2005; Chowdhuri et al., 2015; Richalet and Lhuissier, 2015). In diabetic patients local tissue hypoxia is a common feature (Duennwald et al., 2013). Animal studies have shown that decreased mitochondrial bioenergetic capacity in prediabetic rat hearts may

impair respiratory capacity and reduce basal contractile function and tolerance to acute oxygen deprivation (Essop et al., 2009).

The aim of this study was to examine the chain of events occurring during AHT in healthy subjects of middle and elderly age and patients with prediabetes and to trace the relationship between the individual resistance to hypoxia and shifts of carbohydrate metabolism in the body. For this purpose, we examined some cardiorespiratory and metabolic parameters before, during and after 20 min of acute hypoxic test.

Materials and methods

I. Participants

We enrolled 63 participants, sea level residents of middle and elderly age that observed at Institute of Gerontology. All study participants were divided into two groups depending on carbohydrate metabolism indices: Gr.I (healthy subjects with normoglycaemia) or Gr.II (prediabetic patients). Prediabetes was diagnosed according to the criteria issued by the American Diabetes Association (2014), that is Gr.II included subjects who had impaired fasting glucose (FPG level of 5.6 - 6.9 mmol/l), impaired glucose tolerance (2hr plasma glucose level of 7.8 - 11.0 mmol/l after a 75 grams oral glucose challenge), or their combination. Inclusion criteria were also the absence of infections during the last month, absence of major cardiovascular or respiratory complications. Gr.I consisted of 33 volunteers (11 males, 22 females), mean age, 63,0; range, 44–76. Gr. II assembled 30 patients (9 males, 21 females), mean age, 65,5; range, 44–75. All participants were nonsmokers.

The clinical trial was conducted according to the laws of Ukraine and the principles of the Helsinki Declaration of Human Rights. The program of research, patient information and informed consent forms were approved by the Ethics Committee of Chebotarev Institute of

Gerontology (protocol # 9, June 11, 2013). All subjects received extensive information of the study process, and written informed consent was obtained.

All subjects underwent measurements of several anthropometric variables (Table 1). Both groups did not differ in age, height, weight, body mass index (BMI) and waist circumference (WC).

II. Experimental procedure

All participants were tested in the sitting position in a quiet room at comfortable temperature. Measurement sessions were performed on two different days. On the first day, after 3-day routine hospital diet (250-300 g carbohydrates) and normal physical activity, in the morning, on an empty stomach venous blood was drawn from the median antecubital vein to measure blood lipids, fasting glucose and fasting insulin. After that the standard oral glucose tolerance test (OGTT) was conducted to identify prediabetic carbohydrate metabolism disorders according to (Rydén et al., 2007). The test was performed using 75 grams of glucose in 250 mL of water. Venous blood was drawn at 30, 60 and 120 min after glucose drinking. According to American Diabetes Association/ADA (1997), the glucose tolerance considered normal if plasma glucose level after 120 min of glucose intake was less than 7.8 mmol / L. If the fasting glucose level was less than 7.0 mmol / L, but after 2 hours it was in the range of 7.8 to 11.1 mmol / L, this state was classified as impaired glucose tolerance. In parallel with plasma glucose determination, plasma insulin was also analyzed before and at 120 min after glucose usage.

On the second day the acute hypoxic test (AHT) was performed in the morning, after 2 hours of light breakfast. Firstly venous blood was drawn to measure plasma glucose and insulin at normoxia. Than the baseline cardio-respiratory parameters were measured during 5 min when participants set in a comfortable chair and breathed spontaneously of room air. Arterial oxygen saturation (SaO_2), heart rate (HR) and breathing frequency (f) were recorded

using Patient Monitor "UM 300-12" ("UTAS", Ukraine, http://www.utasco.com). Measurements of systolic (SAD) and diastolic (DAD) blood pressure were conducted by mercury sphygmomanometer Erkameter 3000 (Germany) on brachial artery according to ESH guidelines. After that participants were connected to an open breathing circuit through a mask and breathed during 20 min with standardized hypoxic gas mixture (12 % O_2 and 88% N_2). At 20th min of hypoxic period venous blood was drawn again. Then subjects were switched to room air breathing. During hypoxia and 5 min of recovery period cardio-respiratory parameters were monitored.

III. Blood chemistry analysis

Plasma glucose concentrations were analyzed by glucose oxidase method in normoxic conditions on semi biochemical analyzer BTS-330 using reagents "Glucose", Bio LATEST Lachema Diagnostica. Insulin levels were measured by immunoenzyme method using DRG Insulin ELISA kit (DRG Instruments GmbH, Germany). Insulin resistance was estimated by homeostasis model assessment (HOMA-IR) using the following formulae (Bonora et al., 2000)**:** HOMA-IR = (fasting plasma insulin (μU/mL)×fasting plasma glucose (mmol/L))/ 22,5.

Blood lipids were measured in the fasting state and analyzed using standard methods on the automatic biochemical analyzer «BM Autolab PM 4000/3» (Boehringer Mannheim company). The serum biochemical parameters included total cholesterol (TC), high-density lipoprotein cholesterol (HDLC), low-density lipoprotein cholesterol (LDLC), very-low-density lipoprotein cholesterol (VLDLC) and triglycerides (TG).

IV. Statistical analysis

All data are presented as means ± SD. Statistical data processing performed using "Statistica 6,0 for Windows" (StatSoft, USA) and analyzed using unpaired Student's *t* test.

Linear regression analysis was used to test for association between variables. Statistical significance was defined as a p value ≤0.05.

Results

Table 1 demonstrates that the groups did not differ in age and anthropometric parameters. Although the fasting glucose in both groups was within the normal values, this parameter in Gr. II was significantly higher compared to Gr.I ($p < 0.01$). Fasting plasma insulin did not differ significantly in two groups and was within the normal range (Table 2).

OGTT has shown that in 2 h after 75 g glucose ingestion the plasma glucose increased in Gr.I by 14.7% and in Gr.II by 26.7% exceeding the value of Gr.I by 37% ($p < 0.01$). Plasma 2 h post-OGTT insulin did not change significantly in Gr.I but increased by 270% in Gr. II ($p < 0.01$) and became more than double compared to healthy subjects..

Serum total cholesterol, high-density lipoprotein cholesterol, low- and very-low-density lipoprotein cholesterol and serum triglycerides did not differ in the two groups. However, the HOMA-IR index was significantly higher in the group with prediabetes (Table 3). Thus, subjects of Gr. I meet all the criteria of normal glycaemia and normal glucose tolerance whereas subjects of Gr. II are characterized by impaired carbohydrate metabolism (IFG, IGT or both).

All study participants were subjected to acute hypoxia test. No adverse effects were observed in both groups. In patients with impaired carbohydrate metabolism AHT displayed less effective functioning of the respiratory and cardiovascular system during 20-min hypoxic impact compared to healthy people. Fig. 1 shows that during first two minutes of AHT the arterial oxygen saturation drops equally in both groups, but during next 10 min the rate of SaO_2 decline increases in Gr. II and becomes significantly lower than in Gr. I by the 15^{th} min of the test: in Gr.I it was 85,5 ± 2,4%, while in Gr. II SaO_2 was 81,1 ± 1,3% ($p < 0.05$). This difference maintained up to the end of the test. Moreover, the recovery time was longer in

prediabetic patients: in 5 min after the hypoxic load termination, SaO_2 remained reduced in Gr. II (96,1±0,7%) while in Gr. I it was even somewhat higher that at initial state (99,0±0,2 %).

Complete results of the cardiorespiratory data during AHT are summarized in Table 4. Cardiovascular reactions to hypoxic load did not differ in both groups: at 20th min of AHT a significant increase in heart rate was observed by 7.5% ($p < 0.05$) in Gr. I and by 8.2% ($p < 0.05$) in Gr.II, SBP heightened by 7.8% ($p < 0.05$) and 10.8% ($p < 0.05$), respectively, and DBP increased by 16.8 % ($p < 0.05$) and 15% ($p < 0.05$), respectively. The respiratory rate also changed similarly: there was a significant increase by 37% ($p < 0.01$) in the Gr. I and by 48% ($p<0,01$) in Gr. II. However, at 5 min of the recovery period SBP, heart rate and respiratory rate were significantly higher in patients with prediabetic disorders than in healthy subjects showing lower recovery rate of functions.

Glucose and insulin levels were not significantly changed under 20-min hypoxia in both groups (Table 4). Thus, single bout of breathing with 12% oxygen hypoxic mixture during 20 min did not exert any influence on the carbohydrate metabolism.

The correlation analysis of all participants' data (Gr. I and Gr. II together) revealed the essential relationship between the resistance to acute hypoxia (which was estimated by the degree of SaO_2 drop at 20th min of hypoxic test) and some indices of carbohydrate metabolism as well as the some cardiorespiratory reactions. It was shown that subjects with lower SaO_2 level during AHT had a higher fasting plasma glucose and insulin ($r = -0,83$ and -0,27, respectively; Fig.2, A and C) and higher 2 h post-OGTT glucose and insulin levels ($r = -0,75$ and -0,40, respectively; Fig.2, B and D).

A negative relation between the resistance to hypoxia and reactivity of cardiorespiratory system was also found: the lower the resistance to hypoxia, the higher the respiratory rate and heart rate at rest ($r = -0,42$ and -0,41, respectively; $p < 0.01$) and higher

their gains during the hypoxic load (r = -0,35 and -0,34, respectively; p <0.05). The results indicate the relationship between the degree of arterial hypoxemia during acute hypoxia and the severity of carbohydrate metabolism disorders in humans.

Discussion

The present findings demonstrate that the prediabetic subjects of middle and elderly age have a lower resistance to acute hypoxia compared with people of the same age but without carbohydrate metabolism disorders. This is confirmed by greater decrease in blood oxygen saturation during 20-min hypoxic exposure to moderate hypoxia, longer recovery time, less effective functioning of respiratory and cardiovascular systems (a greater increase of cardiorespiratory reactions during hypoxia on the background of lower drop of SaO_2). Single bout of breathing with 12% oxygen hypoxic mixture during 20 min did not affect significantly the plasma glucose and insulin levels. However, the initial level of these parameters was negatively correlated with the shift of SaO_2 level during hypoxia: the more drop of oxygen saturation, the higher level of fasting and 2 h post-OGTT glucose and insulin were registered.

People with elevated but non-diabetic levels of blood glucose are at risk of progressing to type 2 diabetes mellitus (Hostalek et al., 2015). Increased secretion of insulin initially compensates for the presence of insulin resistance; however, a simultaneous and progressive loss of β-cell mass and β-cell function limits the ability of the pancreas to maintain euglycaemia by increasing insulin secretion (Nathan et al., 2007). In our investigation the subjects of Gr. I meet all the criteria of normal glycemia and normal glucose tolerance whereas subjects of Gr. II have all the characteristics of prediabetes. Although the fasting glucose in both groups was within the normal values, this parameter in Gr. II was significantly higher compared with Gr. I. The groups did not differ in serum blood lipids; however the HOMA-IR index was significantly higher in the group with prediabetes. Two

hours post-OGTT glucose and insulin in prediabetic patients exceeded significantly the values of healthy elderly subjects.

In our investigations initial level of SaO_2 during air breathing was slightly lower (but not statistically significant) in prediabetic patients compared to healthy subjects of middle and elderly age. Respiratory and cardiovascular reactions to hypoxic load did not differ significantly in healthy subjects and individuals with prediabetic disorders (we registered traditional increase in heart rate, breathing frequency and arterial blood pressure), but prediabetic patients showed a significant delay in the recovery of these parameters after hypoxia termination. Moreover, the correlation analysis showed that people with more expressed cardiorespiratory reactions and longer recovery time had greater drop of SaO_2 during hypoxia and elevated levels of fasting and post-OGTT glucose. In other words, hypoxic load revealed significantly greater SaO_2 fall in people with impaired carbohydrate metabolism in spite of greater increase in respiration and heart rate.

In this regard it should be noted that the responses to hypoxia are multidimensional. The study of interaction between glucose and oxygen has focused on the carotid bodies, because they are the primary stimulatory sensors for hypoxia. Previous studies in diabetes showed abnormalities in the control of the autonomic nervous system, blood pressure, microcirculation and respiration (Bergner and Goldberger, 2010; Duennwald et al., 2013; Bernardi et al.,, 2011; Pokorski et al., 2015), particularly a decrease in ventilatory and cardiovascular responses to hypoxia in diabetic patients (Nishimura et al., 1989; Weisbrod et al., 2005; Duennwald et al., 2013). On the other hand, Ward et al. (2007) have shown that hypoglycemia, as well as hyperglycemia, produced an increase in ventilation and in the hypoxic ventilatory response, being the latter accompanied by an increase in circulating counter-regulatory hormones. One of the hypotheses that came out to explain the role of the

CB in glucose homeostasis was the potential of the CB as a glucosensor (Conde et al.,2014; Pokorski et al., 2015).

Anyway, by analogy with the age-related changes of cardiorespiratory function (Korkushko et al., 2005, Richalet and Lhuissier, 2015) we can say that in the development of prediabetes carbohydrate disorders the cardiorespiratory reactions to hypoxia increase but the resistance to lack of oxygen decreases exposing reduced effectiveness of adaptive response.

All subjects, both healthy and with impaired carbohydrate metabolism, tolerated the hypoxic test well as performed in this investigation. Our findings agree with latest research of Goto et al. (2015) confirming that a 2-h exposure to moderate hypobaric hypoxia (comparable to 15.0% O_2) may be beneficial for people with impaired glucose tolerance. However, there are doubts in the literature about the safety of the inhalation of hypoxic gas mixtures. Increasing evidence during the past decade indicates that hypoxia may be directly injurious at the cellular level (Burki and Tetenta, 2014). Hypoxic stimulation can cause adverse changes such as vascular hemodynamic deterioration, increased risk of thrombosis, changes in heart rhythm, development of insulin resistance (Lippi et al., 2007; Sanchis-Gomar et al., 2012). Studies suggest that insulin resistance is also positively associated with obstructive sleep apnea (OSA) which accompanied by intermittent hypoxia (Drager et al., 2011; Kim, 2012, He et al., 2014).

On the other hand, there is a lot of evidence that IHT in the modes which are different from those that are used for experimental OSA modeling, have pronounced positive effect (Xi & Serebrovskaya, 2012; Kolesnik et al, 2012; Urdampilleta et al, 2012; Morishima et al., 2014). In order to achieve greater effect of IHT and avoid adverse effects, the dose of hypoxia must be individually assigned.

Conclusions

In this investigation we have shown that subjects with pronounced symptoms of prediabetes have less resistance to acute hypoxia which is manifested in a greater decrease in blood oxygen saturation during hypoxic exposure, longer recovery time, less effective functioning of respiratory and cardiovascular systems. These patterns of relationship must be considered when assigning the individual modes of IHT. Our next study will focus on the task of how to dose hypoxic load for patients with prediabetes based on the results of an acute hypoxic test.

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Table 1

Anthropometric characteristics of the participants

Groups	Sex (female/male), n/n	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m2)	Waist cm
Gr.I. Healthy	23/11	63,3±10,5	166±8,0	80,5±14,0	28,9±4,0	97,2±10,7
Gr.II. Prediabetes	15/8	61,5±7,3	165±7,5	84,6±12,8	30,8±3,6	98,6±8,2
Significance of differences between groups	NS	NS	NS	NS	NS	NS

Data are mean ± SD; BMI, body mass index; Waist, waist measurements

Table 2

Glucose and insulin blood serum concentrations during oral glucose tolerance test (OGTT)

Groups	Fasting glucose (mmol/L)	2 h post-OGTT glucose (mmol/L)	P fasting vs. 2 h post-OGTT	Fasting insulin (mU/L)	2 h post-OGTT insulin (mU/L)	P fasting vs. 2 h post-OGTT
Gr.I. Healthy	4,64±0,51	5,32±1,24	< 0,05	10,67±4,84	24,75±22,1	NS
Gr.II. Prediabetes	5,76±0,67**	7,30±1,83**	< 0,01	15,63±9,46	57,69±32,7**	< 0,01

Data are mean ± SD. **- p < 0.01 vs. Gr.I.

Table 3

Lipid metabolism data

Groups	TC, mmol/L	HDLC, mmol/L	LDLC, mmol/L	VLDLC, mmol/L	TG, mmol/L	HOMA-IR, units
Gr.I. Healthy	5,16±0,94	1,41±0,29	3,24±0,91	0,52±0,23	1,16±0,50	1,96±1,20
Gr.II. Prediabetes	5,42±1,06	1,36±0,27	3,47±0,95	0,65±0,34	1,46±0,75	3,98±2,46*

Data are mean ± SD. * - $p < 0.05$ vs. Gr.I.

TC, serum total cholesterol; HDLC, serum high density lipoprotein cholesterol; LDLC, serum low density lipoprotein cholesterol; VLDLC, serum very-low-density lipoprotein cholesterol ; TG, serum triglycerides; HOMA-IR, homeostasis model assessment of insulin resistance.

Table 4

Cardio-respiratory and biochemical parameters during acute hypoxic test (12% O_2, 20 min)

Paramerers	Gr.I. Healthy	Gr.II. Prediabetes	P Gr.I vs Gr.II
HR, min^{-1}			
normoxia	68,3±8,01	71,0±6,39	NS
20 min hypoxia	73,4±8,92*	76,8±7,08*	NS
5 min recovery	63,4±7,4	72,4±6,10	<0,05
SBP, mm Hg			
normoxia	128±18,2	130±16,6	NS
20 min hypoxia	138±21,1*	144±22,3*	NS
5 min recovery	122±19,1	139±17,1*	<0,05
DBP, mm Hg			
normoxia	80,4±11,2	78,4±12,1	NS
20 min hypoxia	93,9±10,8*	90,3±9,9*	NS
5 min recovery	76,2±12,8	83,4±10,2	NS
f, min^{-1}			
normoxia	15,9±2,64	15,1±3,26	NS
20 min hypoxia	21,8±5,75**	22,4±5,91**	NS
5 min recovery	16,0±3,28	19,8±3,39*	<0,05
Plasma glucose, mmol/L			
normoxia	4,73±1,14	5,40±1,38	<0,05
20 min hypoxia	4,69±1,08	5,08±1,15	NS
Plasma insulin, mU/L			

normoxia	31,4±30,4	36,5±25,5	NS
20 min hypoxia	21,2±16,3	28,1±20,27	NS

Data are mean ± SD. HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; f, respiration rate

*- $p < 0.0\,5$ vs. normoxia; **- $p < 0.01$ vs. normoxia.

Figure legends

Fig.1. **Arterial blood saturation during acute hypoxic test (breathing with 12% O_2) in healthy subjects and patients with prediabetes**

Data are mean ± m.

Full line – Gr.I; dotted line - Gr. II.

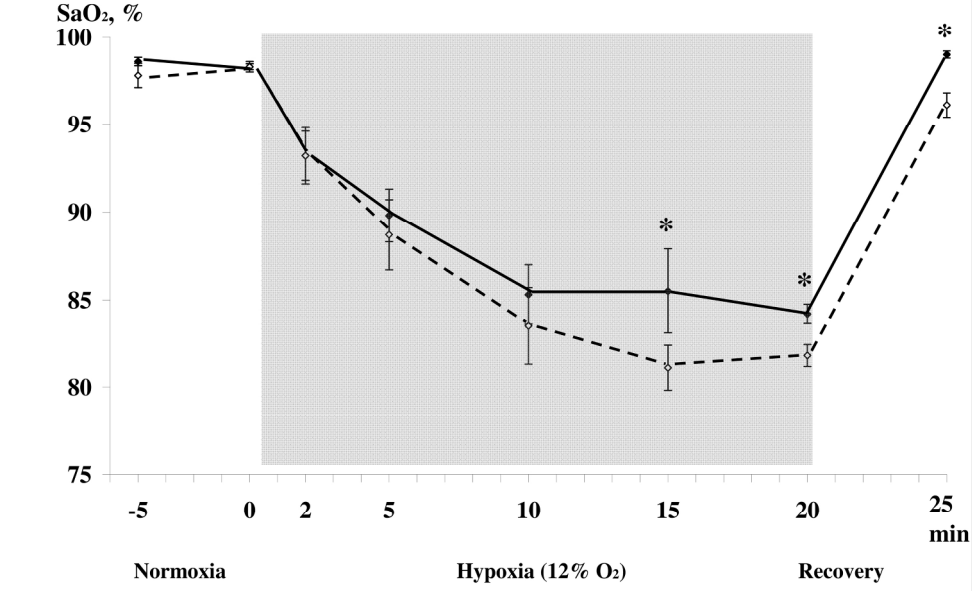
* - $p < 0.05$ vs. Gr.I.

Fig 2. The correlation between arterial blood O_2 saturation during 20-min acute hypoxic test and glucose and insulin concentrations during OGTT in subjects of middle and elderly age.

Correlation analysis is presented to the full set of data (Gr.I + Gr.II).

Upper figures: Correlation between fasting plasma glucose (A), glucose at 120^{th} min of OGTT (B) and SaO_2 at 20^{th} min of AHT (inhalation of 12 % O_2).

Lower figures: Correlation between fasting plasma insulin (C), insulin at 120^{th} min of OGTT (D) and SaO_2 at 20^{th} min of AHT.



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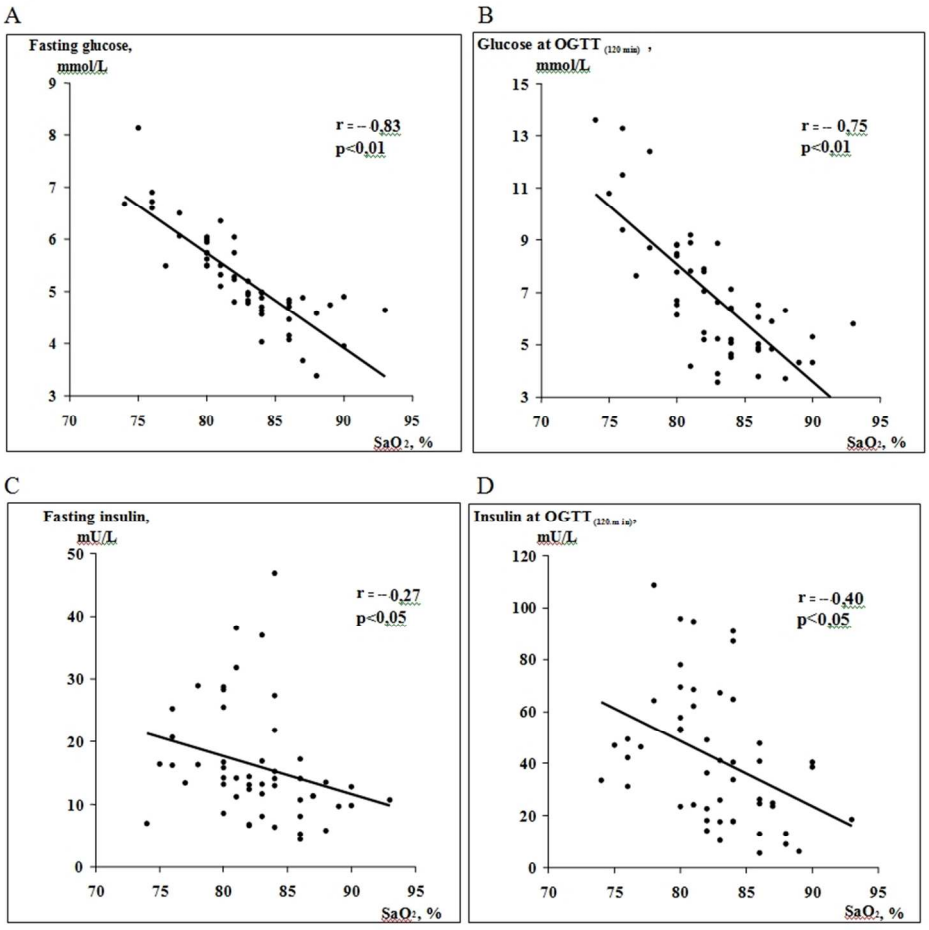

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