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Early View

Research letter

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To the Editor:

There is growing interest in metabolic profiling in pulmonary arterial hypertension (PAH) due to current findings suggesting significant metabolic changes causing pulmonary arterial remodeling and linking PAH to insulin resistance [1]. Such findings may have major impact on future diagnostic and therapeutic strategies for PAH. However, most of the studies have enrolled patients with severe disease whose reduced physical activity may have a profound effect on insulin sensitivity.

Insulin resistance is associated with endothelial dysfunction, impaired angiogenesis, atherosclerosis, cardiomyopathy or myocardial fibrosis [2,3]. In 2009, an elegant study suggested that insulin resistance is quite common in PAH and associated with a poor survival [4]. This was in line with findings in the apoE knock out mouse developing both insulin resistance and PAH [5]. Subsequent studies showed that glucose intolerance is a common feature in idiopathic PAH [6,7] and recently this was explained by a metabolic pattern of lipid-related insulin resistance [8]. Unfortunately, these studies did not investigate whether insulin resistance caused the development of PAH or was a consequence of PAH, e.g. due to loss of physical activity; furthermore, they relied on surrogate markers of insulin resistance rather than direct assessment of insulin sensitivity.

Though surrogate markers of insulin resistance (TG/HDL-C, HbA1c, HOMA-IR) are widely accepted and technically simple to obtain, there is some discussion about their validity for the assessment of insulin sensitivity in individual patients. The technical gold standard is the hyperinsulinemic-euglycemic Botnia clamp investigation. This is an invasive, time-consuming technique requiring dedicated equipment and trained staff [9,10].

We assessed insulin sensitivity by employing simultaneous pairwise matched-control Botnia clamp investigations, and found no indication of insulin resistance in patients with severe idiopathic PAH and preserved physical activity.

Methods

The study was approved by the Institutional Ethical Committee of Medical University of Graz, and registered at https://clinicaltrials.gov/ as ´NCT03584607´. All patients participated after written informed consent and approval of the local Ethics Committee.

Five non-diabetic normal weight IPAH patients and their age-, sex-, and body composition-matched non-diabetic healthy controls were invited to our clinic on the same day, where all investigations on such a patient-control pair were carried out in parallel. The IPAH patients were selected based on interviews showing that they had no major limitations in any daily activity, apart from doing sports. The controls were identified from the Medical University of Graz - Clinical Trials Unit database using the Phoenix Clinical Trial Management System. In all selected controls, pulmonary hypertension was excluded by means of echocardiography. Two control subjects were treated for systemic hypertension, and two for hypothyroidism (Control #1: Candesartan, Levothyroxine; Control #2: Levothyroxine; Control #3: -; Control #4: -; Control #5: Candesartan, Nebivolol, Allopurinol). All IPAH

patients were on PAH therapy (Patient #1 (WHO FC I, Time from diagnosis: 111 months): Amlodipine, Macitentan; Patient #2 (WHO FC II, Time from diagnosis: 228 months): Amlodipine, Macitentan, inhaled Iloprost; Patient #3 (WHO FC II, Time from diagnosis: 178 months): Sildenafil, Macitentan; Patient #4 (WHO FC I, Time from diagnosis: 19 months): Amlodipine; Patient #5 (WHO FC II, Time from diagnosis: 20 months): Macitentan, inhaled lloprost). All participants underwent dual-energy X-ray absorptiometry measurement to estimate whole body adipose tissue distribution. Scan areas were analyzed to determine lean mass, fat mass, bone mineral content, and total body fluid percentage. The subjects received standardized nutrition (Fresenius Kabi) according to the predicted calorie demand (Harris-Benedict equation) on the day before the study, and then were fasted for 20 hours. On the next day dynamic insulin regulation was tested with a combined intravenous glucose tolerance test (IVGTT) and the hyperinsulinemic–euglycemic clamp (Botnia clamp) assessing both insulin secretion and insulin sensitivity[9]. Baseline samples were obtained at standardized times, and subjects were given an intravenous 20% glucose bolus (0.3 g/kg body weight) at the start of the Botnia clamp (0 min). Blood samples for plasma glucose, insulin, and C-peptide were obtained at −10, 0, 2, 4, 6, 8, 10, 20, 30, 40, 50, and 60 min. At 60 min, the hyperinsulinemic–euglycemic clamp was started to determine insulin sensitivity. A priming dose of insulin (3 IU/m^2) followed by an infusion (40 mU/m^2/min) of short-acting human insulin was continuously infused into a peripheral vein for 120 min. Blood glucose level was held constant (clamped at 100±10mg/dl) by intravenous infusion of 20% glucose, using the negative feedback principle. Blood samples for measurement of plasma glucose and insulin concentrations were obtained at 5- and 30-min intervals, respectively, throughout the clamp. Glucose concentrations were measured with the Super GL compact analyzer (Hitado), insulin and C-peptide levels with chemiluminescence on an ADVIA Centaur system (Siemens). Differences between patients and their matched controls were tested with the 2-sided exact test. $P < 0.05$ was considered statistically significant.

Results

We enrolled five pairs of IPAH patients (mPAP: 40±8 mmHg, Cardiac Index: 2.6±0.6 L/min/m^2, PVR: 7.2±3.6 WU, RAP: 5±3 mmHg, PAWP: 8±2mmHg) and their healthy controls (Female sex: 80% vs. 80%, Age: 58.7±8.0 yrs vs. 59.9±7.5 yrs, 6min walking distance: 487.8±59.7 m vs.486.8±69.9 m, NT-proBNP: 327.0±416.0 pg/ml vs. 82.2±40.8 pg/ml, FEV1: 100.6±8.9 %pred vs. 109.7±8.2 %pred, $DLCO_C$SB: 70.4±10.4 %pred vs. 91.7±14.7 %pred (p=0.032)) over a period of 10 months. IPAH patients displayed lower systemic blood pressure (SysBP: 113.6±13.4 mmHg vs. 137.6±10.01 mmHg (p=0.016), DiaBP: 70.4±6.1 mmHg vs. 88.6±9.4 mmHg (p=0.016)) and higher NT-proBNP as compared to their controls but no other significant differences, apart from pulmonary hemodynamics. The two groups were comparable in terms of physical characteristics (BMI: 24.0±2.5 kg/m^2 vs. 25.3±4.4 kg/m^2, Body Fat Percentage: 35.8±5.6 % vs. 34.7±9.9 %, Trunk fat mass/Total fat mass: 0.47±0.1 vs. 0.51±0.1), fasting blood glucose levels (84.4±2.7 mg/dl vs. 83.0±6.1 mg/dl), HbA1c levels (36.4±2.1 mmol/mol vs. 34.4±2.3 mmol/mol) and TG/HDL-C ratio (1.1±0.3 vs. 1.9±1.0). Both groups displayed normal efficacy of glycemic control (HOMA index: 0.84±0.2 vs. 1.09±0.6). The Botnia clamp measurements showed no differences in insulin response ($AUC_{\Delta Insulin}$: 310±156 vs. 367±215, p=0.69; $AUC_{\Delta C\text{-}peptide}$: 26.1±6.9 vs. 32.2±15.1, p=0.69 and disposition $index_{M\ value^*\ AUC\Delta Insulin}$: 3828±2058 vs. 4669±1613, p=0.53) or insulin sensitivity (Figure 1B) in any of the IPAH patients when compared to their healthy controls and also the comparison of the groups showed no significant differences. In

IPAH, the whole-body glucose disposal capacity in response to insulin infusion showed the same characteristics as in healthy controls (Figure 1C).

Discussion

We applied the gold standard Botnia clamp method to evaluate insulin sensitivity in IPAH patients with severely elevated pulmonary arterial pressure but well preserved right ventricular function and normal daily activity. The Botnia clamp determines the metabolic clearance rate of glucose upon infused insulin, and currently represents the most accurate method to assess insulin sensitivity. Decreased insulin sensitivity defines insulin resistance.

In this study we detected no indication of insulin resistance in patients characterized by manifest IPAH but no major limitations in their daily physical activity. This does not rule out the possibility that metabolic dysregulation may occur when the disease causes decreased physical activity and that this dysregulation worsens the pulmonary vascular remodeling. However, our finding speaks against the actual hypothesis that insulin resistance represents an important primary cause for severe remodeling of the small pulmonary arteries leading to idiopathic PAH.

As a limitation, our small cohort does not represent the full range of IPAH patients, and responders to calcium channel blockers have been overrepresented. The application of PAH medications might have influenced insulin sensitivity. We have studied a prevalent population of long-term survivors and results might be different in an incident population. It is possible that patients suffering from more severe disease causing decreased physical activity and overweight may show different results. However, such changes in insulin sensitivity would be considered as secondary effects of the disease and not as an underlying cause of pulmonary hypertension.

In conclusion, this study does not support insulin resistance to be a primary cause of pulmonary vascular remodeling in IPAH.

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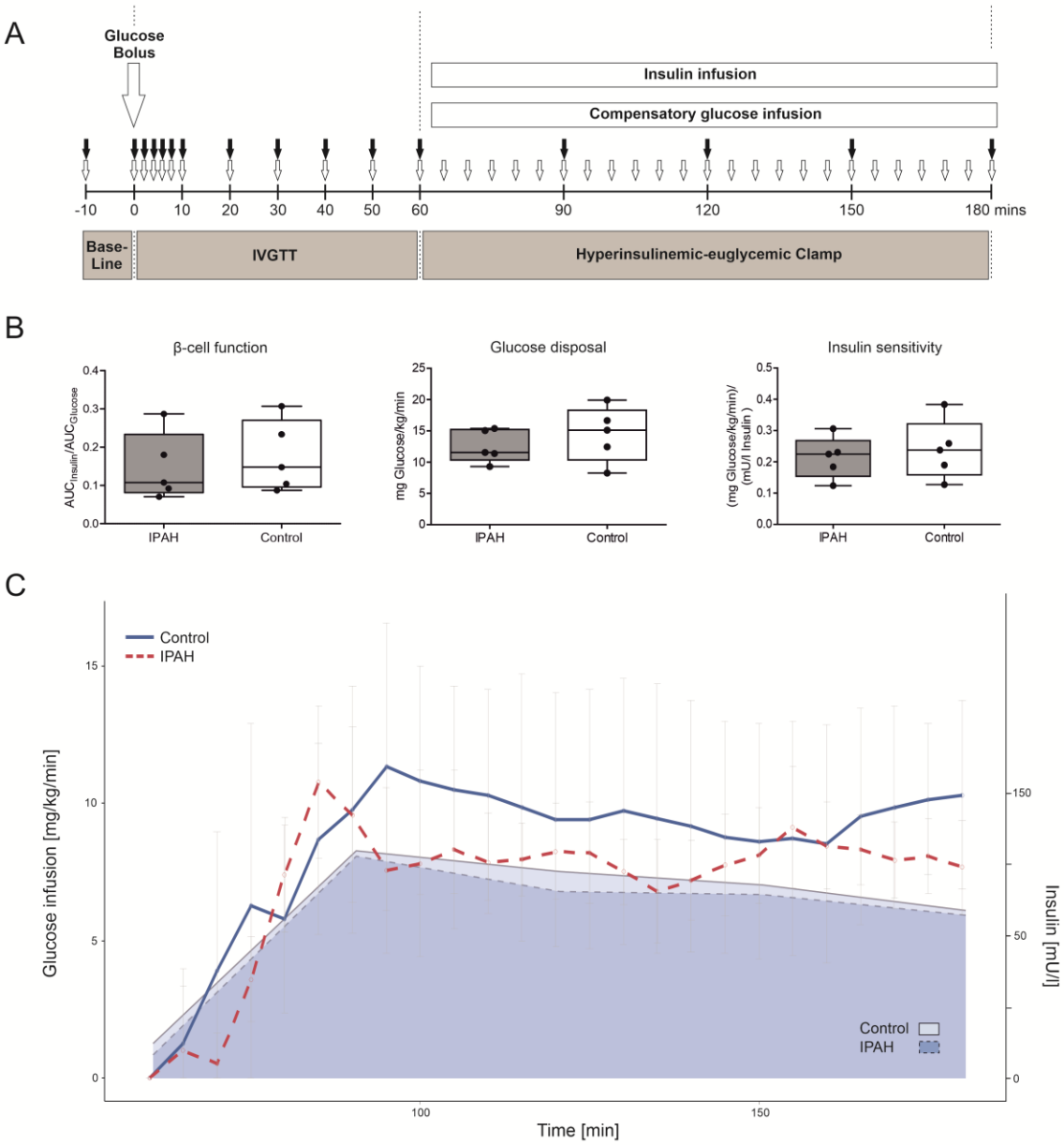
Figure 1.

Insulin sensitivity in pulmonary hypertension.

A) Representative scheme of the Botnia clamp protocol. Small arrows indicate blood sampling for assessing glucose (white) or blood insulin and C-peptide (black) levels. B) B-cell function: calculated as plasma insulin incremental AUC over plasma glucose incremental AUC during the first 10 min of IVGTT. Glucose disposal: or ´M value´, amount of metabolized glucose; calculated by glucose infusion rate per kg lean body weight during steady-state period corrected by plasma glucose space-correction. Insulin Sensitivity: or ´S_I Index´, the quantity of glucose metabolized per unit of plasma insulin concentration, calculated by the steady-state rate of exogenous glucose infusion divided by the insulin concentration during the same time period (mg/kg/min per mU/L). C) Glucose infusion rate in IPAH and control subjects (lines) in relation to their corresponding mean insulin levels (blue areas). n=5.


A
Glucose Bolus
Insulin infusion
Compensatory glucose infusion
-10
0
10
20
30
40
50
60
90
120
150
180 mins
Base-Line
IVGTT
Hyperinsulinemic-euglycemic Clamp
B
β-cell function
Glucose disposal
Insulin sensitivity
IPAH
Control
C
Control
IPAH
Glucose infusion [mg/kg/min]
Insulin [mU/l]
Time [min]