Background: Recent studies have introduced glucose intolerance and insulin resistance (IR) as novel risk factors in patients with pulmonary arterial hypertension (PAH).

Objectives: We aimed to investigate the prevalence of glucose intolerance and IR in patients with PAH and their correlation with functional capacity and prognostic factors.

Patients and Methods: Sixty-nine patients with pulmonary arterial hypertension (class I Pulmonary hypertension in accordance with updated clinical classification of pulmonary hypertension) scheduled for right heart catheterization were enrolled. FBS, HbA1c, lipid profile, pro-BNP and hs-CRP were measured along with a 6-minute walk test (6-MWT) and obtaining demographic, functional and hemodynamic data. Fasting triglyceride to high-density lipoprotein cholesterol ratio (TG/HDL-C) was used as a surrogate of insulin sensitivity. Using published criteria, HbA1c ≤ 5.9% defined as normal, 6.0-6.4% as glucose intolerance, and ≥ 6.5% as diabetes. All patients were followed for a year regarding development of any cardiovascular event (mortality and/or hospitalization).

Results: In total, 76.8% of patients were female: 61% of them had idiopathic PAH, 33% Eisenmenger syndrome, and 6% PAH secondary to a connective tissue disease. With respect to TG/HDL-C, 43.5% of patients had IR and 47.8% of patients had HbA1c > 6. There was no difference between IR and insulin sensitive (IS) group or glucose intolerance and sensitive group regarding NYHA class, 6MWT, Pro BNP, hs-CRP and hemodynamic data and there was no correlation between IR or glucose intolerance and any event.

Conclusions: Unrecognized glucose intolerance and IR are common in PAH. However, further studies are needed to show whether glucose or insulin dysregulation plays any role in PAH pathogenesis or it is secondary to advanced PAH.

Keywords: Insulin Resistance; Pulmonary Arterial Hypertension; Glucose Intolerance

1. Background
Pulmonary arterial hypertension (PAH) is a progressive and debilitating disorder characterized by increase in pulmonary vascular resistance (PVR) leading to right sided heart failure and death (1-3). The pathophysiology of PAH is complex and multiple pathways and gene mutations are involved in its development and progression. This underlies the importance of ongoing investigations to explore novel targets and disease modifiers. Recent humans and animals studies have demonstrated an association between insulin resistance (IR) and glucose intolerance with PAH (2-5). According to the American Diabetes Association Expert Committee, elevated HbA1c identifies patients with abnormal glucose metabolism (6). While measurement of HbA1c does not quantify IR, some evidences presented that the ratio of serum triglyceride to high density lipoprotein cholesterol (TG/HDL-c) could provide a surrogate for IR in healthy subjects and patients with PAH (2, 7-9). This test has been shown to be as sensitive and specific as fasting insulin in determining IR in individuals without diabetes.

2. Objectives
In the present study, we aimed to evaluate the prevalence of IR and unrecognized glucose intolerance by testing both TG/HDL-c ratio and HbA1c in patients with PAH and to correlate them with disease severity, functional status and prognostic factors of patients.

3. Patients and Methods
3.1. Patient Selection
Sixty-nine consecutive patients with the World Health Organization Category I pulmonary arterial hypertension (PAH) scheduled for right heart catheterization for evaluation of pulmonary hemodynamics for the first time or follow-up were included between March to December 2011. The exclusion criteria comprised, established diabetes mellitus and hyperlipidemia, hemoglobinopathy, hemolytic anemia, thalassemia, overt fluid retention, decompensated right sided heart failure, significant left ventricular dysfunction (left ventricle ejec-
tion fraction less than 45- 50%), NYHA class of IV and inability to perform 6-minute walk test (6-MWT). Patients taking prednisolone or other oral corticosteroids were also excluded.

Primary evaluation was performed and clinical history obtained from all the patients, and detail demographic data and the New York Heart Association (NYHA) classification were recorded. Exercise tolerance and functional performance of patients was assessed by 6-MWT according to the protocol of Guyatt and colleagues (10).

3.2. Laboratory Examinations

Whole blood was collected from all study participants after 12-14 hours overnight fasting. All the blood analyses were performed at our laboratory on the day of blood collection. Fasting blood sugar (FBS) was measured using enzymatic colorimetric method with glucose oxidase. Total cholesterol was assayed using enzymatic colorimetric method with cholesterol oxidase and cholesterol esterase. Triglyceride (TG) was assayed using an enzymatic colorimetric method with glycerol phosphate oxidase. HDL-C was measured after precipitation of the apolipoprotein B-containing lipoproteins with phosphotungstic acid. All analyses were performed using Pars Azmon kits (Pars Azmon Inc., Tehran, Iran) and a Selectra 2 autoanalyzer (Vital Scientific, Spankeren, The Netherlands). Glycosylated hemoglobin (HbA1c) was measured using high performance liquid chromatography (HPLC) by standardized laboratory protocol using a method certified by the National Glycohemoglobin Standardization Program. Serum Pro-BNP was analyzed using the ELISA (Biomedia-Corp., Bratislava-Slovakia) and serum CRP levels were measured by slide agglutination method and immunoturbidimetry using a CRP latex kit (Bionic-USA) for each sample.

3.3. Right Heart Catheterization

Right heart catheterization was performed by standard method in all patients using 7F balloon-tipped, double lumen pulmonary artery (PA) catheters in the catheterization laboratory. All the measurements were obtained with patients at rest in the supine position, breathing room air. The pressures were all averaged in three consecutive heart beats at end expiration. The following variables were measured for each patient: pulmonary capillary wedge pressure (PCWP); systolic, diastolic, and mean pulmonary artery pressure; systolic and end-diastolic right ventricular (RV) pressure; mean right atrial pressure; mixed venous oxygen saturation and cardiac output using the Fick technique.

The study population was subsequently followed for a year for survival outcomes (death and hospitalization due to exacerbation of PAH or right sided heart failure). The study was reviewed and approved by the ethics committee at Rajaie Cardiovascular Medical and Research Center, and written informed consent was obtained from all the patients.

3.4. Statistical Analysis

Patients were defined to have a normal status, glucose intolerance or diabetes based on HbA1c measures and having insulin resistance or sensitivity based on the TG/HDL-c ratio. Table 1 depicts definitions used for defining the study subgroups. IBM SPSS Statistics 19.0 for Windows (IBM Corp., Armonk, NY, USA) was used for all the statistical analyses. All data were initially analyzed using the Kolmogorov-Smirnov test to assess normality.

Categorical variables were presented as numbers and percentages and quantitative variables as mean (standard deviation). Categorical data were compared by chi-square test and quantitative variables by Student t-test or Mann-Whitney test, as appropriate. For comparative analysis of quantitative variables between HbA1c or Insulin resistance subgroups, one-way ANOVA was used. Relationships were assessed using Pearson or Spearman correlation coefficient depending on their distribution. A P value < 0.05 was considered statistically significant.

| Table 1. Definitions for Glucose Intolerance and Insulin Resistance |
|------------------------|---------------------------------|
| **Term**               | **Definition**                  |
| Glucose intolerance    | HbA1c less than or equal to 5.9% was considered normal. Glucose intolerance (GI), was defined as HbA1c 6.0-6.4%, and frank diabetes as HbA1c ≥ 6.5% in accordance with the latest guideline released by the American Diabetes Association Expert Committee (6). |
| Insulin resistance     | Based on several studies (11, 12), we defined an individual as insulin resistant (IR) when TG/HDL-C ratio was greater than 3.0, and insulin sensitive (IS) when TG/HDL-C ratio was less than 2.0. |
| The Triglyceride (TG) to High-Density Lipoprotein (HDL-C) ratio (TG/HDL-C) was used as a surrogate measure of insulin resistance profile. |
| Obesity                | Subjects with BMI ≥ 25 kg/m^2 and BMI ≥ 30 kg/m^2 were considered overweight and obese, respectively (6). |
4. Results

4.1. Clinical, Laboratory and Hemodynamic Characteristics of Patients

Sixty-nine new and returning patients with WHO group I PAH, including 16 (23%) men and 53 (76%) women were enrolled. The mean age was 35 (13.3) years (range; 14-74 years). Idiopathic PAH was the main diagnosis (61%) and the remaining 33% and 6% were diagnosed as Eisenmenger syndrome and PAH secondary to connective tissue diseases, respectively. At the time of clinical evaluation, 41% of patients had NYHA class I-II and II and 59% had NYHA class II-III and III. No patient was completely asymptomatic (NYHA class I). Table 2 depicts the clinical and laboratory characteristic of patients and Table 3 shows their right heart catheterization results.

4.2. HbA1c Level in PAH and Presence of Glucose Intolerance

In Sixty-six patients (52.2%), HbA1c was within the normal limits (≤ 5.9%). Considering the HbA1c measures, 13 patients (18.8%) showed glucose intolerance (HbA1c ≥ 6-6.4%) and 20 patients (29%) had overt DM (HbA1c ≥ 6.5%). Considering FBS, 7% of patients had a FBS ≥ 126 mg/dL and the mean of FBS was significantly higher among patients with HbA1c more than 6% (139 ± 52.7 mg/dL versus 95.7 ± 16.7 mg/dL, P < 0.001). HbA1c was not significantly different between men and women or different diagnosis groups. Patients in DM group were older than others, but this difference was not statistically significant (37.4 ± 14.3 versus 34.1 ± 14.5 and 33.3 ± 12.5 in patients with and without glucose intolerance, respectively).

Regarding body weight, there was no significant difference between three HbA1c groups; however PAH patients with HbA1c more than 6.5% had a slightly higher BMI than the two others (26.7 ± 4 in HbA1c ≥ 6.5% versus 24.1 ± 3.7 and 23.9 ± 4 in patients with and without glucose intolerance respectively, P = 0.05). There was no association between NYHA class and HbA1c. The mean of pro BNP, 6-MWT and CRP were not different between different HbA1c groups (Table 4).

There was no difference between different HbA1c groups with respect to hemodynamic measures (Table 4). The bivariate analyses showed no correlation between HbA1c and ProBNP, 6-MWT and hemodynamic measures (r between 0.001 and 0.24 with all P values more than 0.05); however, there was a weak but significant correlation between CRP and HbA1c (r = 0.24, P = 0.04). The ratio of TG/HDL showed no difference between patients with or without glucose intolerance (2.9 versus 3, P = 0.9).

4.3. Survival Outcomes and Glucose Intolerance

All patients were followed for a year for combined event including death or hospitalization due to PAH exacerbation and no patient was missed during a year of follow-up. During follow-up, 17 (24.6%) patients were hospitalized due to PAH exacerbation and six (8.7%) patients died. Among those who died, four patients had a NYHA class of II-III and III and two patients had a NYHA class of I-II and II (not statistically significant).

The mean of HbA1c was not different between patients with and without events (5.7% ± 11 versus 5.6% ± 12, P > 0.05) and there was no association between HbA1c and all events. However as shown in Table 6, FBS was significantly higher in patients with an event (P < 0.001). Multivariable logistic regression model was applied to investigate the associations between the occurrence of combined events and HbA1c, adjusted for other predictors. No significant association was observed between HbA1c and event (β = -0.45, P = 0.1, OR [CI 95%]: 1.6[0.8-3.1]).

Table 2. Descriptive Statistics in the Study Participants (N = 69) a,b

| Characteristic       | Descriptive Index |
|----------------------|-------------------|
| Age, y               | 35 ± 13           |
| Gender               |                   |
| Female               | 53                |
| Male                 | 16                |
| Weight, kg           | 68 ± 12           |
| Height, cm           | 165 ± 9           |
| BMI, kg/m²           | 25 ± 4            |
| Diagnosis            |                   |
| IPAH                 | 42 (61)           |
| Eisenmenger          | 23 (33)           |
| CTD                  | 4 (6)             |
| Pharmacotherapy      |                   |
| PDEI5                | 36 (52)           |
| ERA                  | 1 (1.4)           |
| CCB                  | 7 (10)            |
| PDEI5 + ERA          | 25 (36)           |
| NYHA-Class           |                   |
| I, I to II           | 28 (41)           |
| II, II to III        | 41 (59)           |
| hs-CRP, mg/dL        | 7 ± 5             |
| NT-Pro BNP, ng/dL    | 208 ± 4708        |
| 6-MWT, m             | 263 ± 1200        |
| FBS, mg/dL           | 103 ± 31          |
| HbA1C, %             | 5.7 ± 1.1         |
| CHOL, mg/dL          | 151 ± 43          |
| HDL-c, mg/dL         | 40 ± 10           |
| LDL-c, mg/dL         | 93 ± 32           |
| TG, mg/dL            | 116 ± 70          |
| TG/HDL-c ratio       | 2.98 ± 1.48       |

a Data are presented as mean ± SD for quantitative and count (%) for qualitative variables.

b Abbreviations: BMI, body mass index; CCB, calcium channel blocker; CTD, connective tissue disease; CHOL, serum cholesterol level; ERA, endothelin receptor antagonist; FBS, fasting blood sugar; HDL-c, high density lipoprotein cholesterol; hs-CRP, high sensitive C-reactive protein; HbA1c, glycosylated hemoglobin; IPAH, idiopathic pulmonary artery hypertension; LDL-c, low density lipoprotein cholesterol; NYHA, New York heart association; NT-Pro BNP, N-terminal pro brain natriuretic peptide; PDEI-5, phosphodiesterase inhibitor-5; 6-MWT, six-minute walk test; TG, triglyceride.
4.4. TG/HDL Ratio in PAH and Presence of Insulin Resistance

Regarding the ratio of TG/HDL to determine insulin resistance (IR), 16 (23.2%) patients had a ratio less than 2 [insulin sensitive (IS) group]. In 30 (43.5%) patients, the ratio was more than 3 (IR group). The rest of patients (33.3%) had a ratio between 2-3 (in determinant). The ratio was not different between men and women and there was no statistically significant difference between different diagnostic groups. IR group were older than IS group, but the difference was not statistically significant (37.3 ± 13.5 versus 32.5 ± 15, P > 0.05). The weight was identical in both IR and IS groups (65.7 ± 12.6 versus 65.2 ± 11.8); however, BMI was a little higher but not statistically significant in IR than IS (24.4 ± 4 versus 23.5 ± 4.5). With respect to functional class, there was no association between NYHA function class and insulin resistance in patients with PAH.

The mean of pro BNP, 6-MWT, hs-CRP and hemodynamic measures were not different between different groups regarding the TG/HDL ratio (Table 5). There was no correlation between pro BNP, 6-MWT, hs-CRP and hemodynamic measures and TG/HDL ratio even after excluding patients with overt diabetes mellitus based on HbA1 level (r between 0.01 to 0.3 with P value more than 0.05 for all variables). There was only a weak correlation between hs-CRP and TG/HDL ratio (r = 0.25, P = 0.04). There was no difference in mean of HbA1c between PAH patients with or without IR (5.7 ± 1.2 versus 5.4 ± 0.95, P = 0.3).

4.5. Survival Outcomes and Insulin Resistance

TG/HDL ratio was not different between patients with or without event and there was no association between IR and mortality, hospitalization and all events; the results were the same after excluding patients with overt diabetes mellitus. After adjustment for other predictors, no significant association was observed between TG/HDL and event in multivariable logistic regression model (β = -0.05, P = 0.8, OR [CI 95%]: 1.05[0.6-1.7]).

4.6. Comparison of PAH Subgroups

The comparison between three diagnostic groups of PAH showed that regardless of significant differences in hemodynamic findings, 6-MWT and pro BNP, there was no statistically difference in HbA1c and TG/HDL ratio between different diagnoses. The results are presented in Table 7.

Table 3. Hemodynamic Measures of Study Participants (n = 69) a

| Hemodynamic Measures | Mean ± SD | Range (Min-Max) |
|----------------------|-----------|-----------------|
| RAP, mm Hg           | 15 ± 10   | 1-65            |
| SPAP, mm Hg          | 90 ± 25   | 40-150          |
| DPAP, mm Hg          | 41 ± 16   | 13-75           |
| Mean PAP, mm Hg      | 57 ± 18   | 22-93           |
| PCWP, mm Hg          | 10 ± 2    | 4-17            |
| CO, L/min            | 4 ± 1     | 2-7             |
| CI, L/m²/min         | 2.38 ± 0.7| 1.4-4           |
| PVR, wood unit       | 9.5 ± 6.7 | 2.2-43.5        |

| Abbreviations: CI, cardiac index; CO, cardiac output; DPAP, diastolic pulmonary artery pressure; HbA1c, glycosylated hemoglobin; hs-CRP, high sensitive C-reactive protein; NT-Pro BNP, N-terminal pro brain natriuretic peptide; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; SD, standard deviation; SPAP, systolic pulmonary artery pressure.  

Table 4. Comparison of Predictors and Hemodynamic Measures Between Groups of Glucose Intolerance a,b

| HbA1c group | Normal (n=36) | Glucose Intolerance (n = 13) | Diabetes Mellitus (n= 20) | P value |
|-------------|---------------|-----------------------------|---------------------------|---------|
| hs-CRP, mg/dL | 6 ± 3         | 7 ± 3                       | 9 ± 7                     | 0.07    |
| NT-proBNP, ng/dL | 2852 ± 6192  | 1019 ± 1035                 | 1119 ± 2067               | 0.3     |
| 6-MWT, m    | 256 ± 126     | 260 ± 97                    | 282 ± 126                 | 0.7     |
| RAP, mm Hg  | 16 ± 12       | 13 ± 4                      | 16 ± 9                    | 0.7     |
| SPAP, mm Hg | 93 ± 27       | 92 ± 24                     | 82 ± 18                   | 0.3     |
| DPAP, mm Hg | 44 ± 17       | 42 ± 14                     | 35 ± 14                   | 0.2     |
| Mean PAP, mmHg | 60 ± 19     | 59 ± 18                     | 50 ± 15                   | 0.1     |
| PCWP, mm Hg | 10 ± 2        | 10 ± 2                      | 11 ± 3                    | 0.6     |
| CO, L/min   | 4 ± 1         | 4 ± 1                       | 4 ± 1                     | 0.7     |
| CI, L/m²/min| 2.44 ± 0.82   | 2.24 ± 0.59                 | 2.35 ± 0.66               | 0.7     |
| PVR, Wood unit | 9.6 ± 7.3    | 9.6 ± 6.9                   | 9.3 ± 5.7                 | 0.9     |

a Data are presented as mean ± SD.  
b Abbreviations: CI, cardiac index; CO, cardiac output; DPAP, diastolic pulmonary artery pressure; HbA1c, glycosylated hemoglobin; hs-CRP, high sensitive C-reactive protein; NT-Pro BNP, N-terminal pro brain natriuretic peptide; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; SD, standard deviation; SPAP, systolic pulmonary artery pressure; 6-MWT, six-minute walk test.
Table 5. Comparison of Predictors and Hemodynamic Measures Between Groups of Insulin Resistance\textsuperscript{a,b}

|                        | Insulin Sensitive (n = 30) | Undetermined (n = 23) | Insulin Resistance (n = 16) | P value |
|------------------------|---------------------------|-----------------------|-----------------------------|---------|
| hs-CRP, mg/dL          | 7 ± (3)                   | 6 ± 3                 | 8 ± 6                       | 0.3     |
| NT-proBNP, ng/dL       | 1573 ± 4603               | 3317 ± 6294           | 1302 ± 3106                 | 0.3     |
| 6-MWT, m               | 261 ± 129                 | 248 ± 121             | 276 ± 116                   | 0.7     |
| RAP, mm Hg             | 15 ± 9                    | 19 ± 13               | 13 ± 7                      | 0.1     |
| SPAP, mm Hg            | 92 ± 22                   | 94 ± 25               | 85 ± 25                     | 0.4     |
| DPAP, mm Hg            | 43 ± 17                   | 42 ± 16               | 38 ± 16                     | 0.5     |
| Mean PAP, mm Hg        | 59 ± 18                   | 61 ± 20               | 53 ± 17                     | 0.2     |
| PCWP, mm Hg            | 10 ± 1                    | 11 ± 2                | 10 ± 2                      | 0.2     |
| CO, L/min              | 4 ± 1                     | 4 ± 1                 | 4 ± 1                       | 0.09    |
| CI, L/m\(\text{m}^2\)/min | 2.43 ± 0.69              | 2.02 ± 0.62           | 2.62 ± 0.75                 | 0.09    |
| PVR, Wood unit         | 9.5 ± 5.1                 | 9.8 ± 5.5             | 9.2 ± 8.3                   | 0.9     |

\textsuperscript{a} Data are presented as mean ± SD.
\textsuperscript{b} Abbreviations: CI, cardiac index; CO, cardiac output; DPAP, diastolic pulmonary artery pressure; HbA1c, glycosylated hemoglobin; hs-CRP, high sensitive C-reactive protein; NT-proBNP, N-terminal pro brain natriuretic peptide; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; SD, standard deviation; SPAP, systolic pulmonary artery pressure; 6-MWT, six-minute walk test.

Table 6. Associations Between the Predictors and Study Combined Events During Follow-up\textsuperscript{a,b}

\begin{tabular}{|l|c|c|c|}
\hline
                        & No (n = 46) & Yes (n = 23) & P value \\
\hline
FBS, mg/dL             & 97 ± 14     & 116 ± 48    & < 0.001     \\
HBA1C, %               & 5.7 ± 1.1   & 5.6 ± 1.2   & 0.6         \\
CHOL, mg/dL            & 148 ± 44    & 156 ± 39    & 0.7         \\
HDL, mg/dL             & 40 ± 11     & 39 ± 9      & 0.4         \\
LDL, mg/dl             & 90 ± 33     & 98 ± 30     & 0.6         \\
TG, mg/dl              & 118 ± 81    & 110 ± 44    & 0.5         \\
hs-CRP, mg/dL          & 7 ± 5       & 7 ± 4       & 0.7         \\
NT-proBNP              & 1074 ± 1828 & 3863 ± 7446 & < 0.001    \\
6-MWT, m               & 266 ± 116   & 258 ± 129   & 0.1         \\
TG/HDL ratio           & 3 ± 1.56    & 2.94 ± 1.34 & 0.8         \\
\hline
\end{tabular}

\textsuperscript{a} Data are presented as mean ± SD.
\textsuperscript{b} Abbreviations: CHOL, serum cholesterol level; FBS, fasting blood sugar; HDL-c, high density lipoprotein cholesterol; hs-CRP, high sensitive C-reactive protein; HbA1c, glycosylated hemoglobin; NT-proBNP, N-terminal pro brain natriuretic peptide; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; SD, standard deviation; SPAP, systolic pulmonary artery pressure; 6-MWT, six-minute walk test; TG, serum triglyceride level.

Table 7. Comparison of Prognosis Predictors, Hemodynamic Measures and Insulin Resistance Predictors Between Groups of Diagnosis\textsuperscript{a,b}

|                        | Idiopathic PAH (n = 42) | Eisenmenger Syndrome (n = 21) | Connective Tissue Disease (n = 4) | P value |
|------------------------|-------------------------|-------------------------------|----------------------------------|---------|
| hs-CRP, mg/dL          | 8.5 ± 5.3               | 5.5 ± 3                       | 6.6 ± 4.5                        | 0.06    |
| NT-proBNP, ng/dL       | 1703 ± 3822             | 1186 ± 2168                   | 10016 ± 13181                    | 0.01    |
| HbA1c, %               | 5.7 ± 1.1               | 5.6 ± 1.1                     | 5.3 ± 1.3                        | 0.6     |
| TG/HDL ratio           | 3 ± 1.5                 | 2.7 ± 1.4                     | 3.5 ± 1.9                        | 0.5     |
| 6-MWT, m               | 295 ± 115               | 222 ± 113                     | 179.5 ± 97                       | 0.02    |
| RAP, mm Hg             | 16.1 ± 11.6             | 14.5 ± 5.7                    | 11 ± 9.7                         | 0.5     |
| SPAP, mm Hg            | 86 ± 22                 | 98.8 ± 27                     | 73.3 ± 9.4                       | 0.05    |
| DPAP, mm Hg            | 38.2 ± 15.2             | 47.5 ± 16.5                   | 30 ± 10                          | 0.01    |
| Mean PAP, mm Hg        | 54.5 ± 17.4             | 63.8 ± 19                     | 44.7 ± 10.5                      | 0.05    |
| PCWP, mm Hg            | 10.3 ± 2                | 10.7 ± 1.4                    | 10 ± 4                           | 0.6     |
| CO, L/min              | 4.1 ± 1.4               | 4.1 ± 1.3                     | 4 ± 1.3                          | 0.9     |
| CI, L/m\(\text{m}^2\)/min | 2.4 ± 0.7              | 2.3 ± 0.7                     | 2.4 ± 0.9                        | 0.7     |
| PVR, Wood unit         | 8.7 ± 5.5               | 11.1 ± 8.7                    | 7.7 ± 0.7                        | 0.3     |

\textsuperscript{a} Data are presented as mean (SD).
\textsuperscript{b} Abbreviations: CI, cardiac index; CO, cardiac output; DPAP, diastolic pulmonary artery pressure; HbA1c, glycosylated hemoglobin; HDL-c, high density lipoprotein cholesterol; hs-CRP, high sensitive C-reactive protein; NT-proBNP, N-terminal pro brain natriuretic peptide; PCWP, pulmonary capillary wedge pressure; PVR, pulmonary vascular resistance; RAP, right atrial pressure; SD, standard deviation; SPAP, systolic pulmonary artery pressure; 6-MWT, six-minute walk test; TG, serum triglyceride level.
5. Discussion

The relationship of insulin resistance and glucose intolerance with PAH has been suggested in recent animal and human studies. Pulmonary hypertension is significantly more prevalent among patients with type II diabetes mellitus independent of hypertension, ischemic heart diseases and heart failure (3). Some epidemiological studies showed a high prevalence of IR in patients with PAH (2, 3). Although routine assessment of IR is difficult, HbA1c and the ratio of TG/HDL have been used as surrogates for IR in patients with PAH (2, 3). In the present study, we used both tests to evaluate IR in patients with class I of PAH like previous reports (2, 3) the prevalence of IR was higher than general population in our study population (43.5% had TG/HDL-c ratio ≥ 3 and 47.8% had HbA1c ≥ 6%). The data represented by the National Survey of Risk Factors for Non-Communicable Diseases of Iran indicated that the prevalence of type 2 diabetes in Iranian adults younger than 65 years was 7.7% (14). In our study, patients with higher HbA1c had a slightly higher BMI, but there was no difference in BMI between IR and IS groups based on TG/HDL ratio, indicating that obesity alone was not responsible for IR.

Zamanian et al. (2) evaluated TG/HDL ratio in 81 female patients with PAH and showed for the first time that a TG/HDL ratio more than three is more prevalent in female patients with PAH than general population (45.7% versus 21%). Pugh et al. (3) assessed HbA1c in 41 patients with PAH and 56% and 15% of their study population had HbA1c more than 6% and 6.5% respectively, a prevalence higher than general population. In our study, NYHA class, 6-MWT, and hemodynamics did not differ between IR and IS PAH groups. Similarly, Zamanian et al. and Pugh et al. found no significant correlation between IR and NYHA class, 6-MWT and hemodynamic measures in patients with PAH. However, the presence of IR was associated with poorer 6-month event-free survival in Zamanian et al. (2) study, which is different from our results and Pugh et al. report. Like Pugh et al. (3) report, we did not find any difference in combined event between IR and IS group. One possible explanation for this finding is that most of our patients were recently symptomatic and PAH was newly diagnosed for them, so they were in early stages of their illness and it may be needed to follow them for a longer duration.

Surprisingly, despite high prevalence of IR by the two methods, we found no correlation between HbA1c and TG/HDL-c ratio. There is conflicting data regarding the association between HbA1c and TG/HDL-c ratio in patients with type 2 diabetes, while some studies showed no correlation between HbA1c and TG/HDL-c ratio (15), some of them suggested a significant correlation (16) indicating no general consensus on this issue and it needs to be addressed more in patients with PAH.

High prevalence of insulin resistance in patients with PAH suggests a link between glucose dysregulation and PAH. It is not exactly known whether these abnormalities in glucose/insulin metabolism are a consequence of PAH and a marker of severe pulmonary vascular disorder or if there is a causative relation with the disease and potential development of PAH. Lopez-Lopez et al. (4) showed marked endothelial dysfunction in pulmonary artery of diabetic rats. Hansmann et al. (17) in an experimental model of insulin resistance concluded that insulin resistance, low plasma adiponectin levels, and deficiency of apo E could be considered as risk factors of PAH and PAH can be reversed in animal models by activation of peroxisome proliferator-activated receptor γ. West et al. (18) assessed the association between bone morphogenic protein receptor type 2 (BMPR2) mutation and IR and found that activation of BMPR2 mutation in vivo is associated with early insulin resistance and blood glucose homeostasis dysfunction and insulin resistance may contribute to disease progression and probably not just a bystander or marker of pulmonary vascular disease. Specifically, insulin resistance predates the development of pulmonary vascular disease, exacerbation through high-fat diet worsens the pulmonary vascular phenotype (18). On the other hand, Moral-Sanz et al. (5) analyzed pulmonary vascular function in insulin resistant obese rats and found that obese rats did not show any of the characteristic features of pulmonary hypertension in contrast to other mice models.

IR is a common finding in PAH which highlights a need for heightened awareness of occult glucose intolerance in this setting. Considering the association of low physical activity and IR (19), the importance of non-pharmacologic strategies with exercise based rehabilitation, lifestyle modification and weight loss could be increased in the management of pulmonary hypertension. Finally, our results are somehow in favor of previous studies suggesting a relationship between insulin resistance and pulmonary arterial hypertension. Although we could not find any difference in NYHA class, prognostic factors, hemodynamics and combined event between our IR and IS PAH groups, FBS was significantly higher in patients with combined event, suggesting a role of glucose intolerance in the prognosis of these patients. Therefore, there are many unanswered questions regarding the causal association of insulin resistance and pulmonary hypertension and our findings suggested that IR is not solely a result of the severity of illness in patients with PAH and cannot be simply considered as a disease modifier; further investigations are necessary for clarification of this issue.

In conclusion, clinicians should be more aware of glucose intolerance in patients with PAH and carefully screen DM in them. It is recommended for direct measurement of IR in patients with PAH and experimental models to determine temporal association of insulin resistance and PAH and the role of IR in the pathophysiology of PAH. Clinical trials should be performed to assess whether interventions targeting IR could be beneficial for patients with PAH.
5.1. Limitations
We used TG/HDL-C as a surrogate of insulin resistance based on previous studies in patients with PAH (2) and general population. However, IR markers should be validated in patients with PAH. Furthermore, most of our patients received different PAH therapies with unknown effect on IR markers. It would be more precise to assess untreated PAH patients or to directly measure IR. As we did not measure waist circumference, we could not conclude regarding the role of central obesity. However, IR and IS groups did not show any difference in BMI.

Acknowledgements
The authors would like to thank Mr. Farshad Amouzadeh for his kind helps in English editing and preparation of the manuscript.

Authors’ Contributions
Nasim Naderi: conception and designing the study, collecting data, analysis and interpretation of data, drafting of the manuscript; Pedram Boobejame: collecting data, interpretation of data; Hooman Bakhshandeh: statistical analysis of data and revising of the manuscript; Ahmad Amin: collecting and interpretation of data; Sepideh Taghavi: collecting and interpretation of data; Majid Maleki: interpretation of data and revising of the manuscript.

Funding/Support
This research project was financially supported by Rajae Cardiovascular Medical and Research Center.

References
1. Galie N, Hoeper MM, Humbert M, Torbicki A, Vachiery JL, Barbera JA, et al. Guidelines for the diagnosis and treatment of pulmonary hypertension: the Task Force for the Diagnosis and Treatment of Pulmonary Hypertension of the European Society of Cardiology (ESC) and the European Respiratory Society (ERS), endorsed by the International Society of Heart and Lung Transplantation (ISHLT). *Eur Heart J*. 2009;30(20):2493-537.
2. Zamanian RT, Hansmann G, Snoon S, Lilienfeld D, Rapoport KM, Reaven GM, et al. Insulin resistance in pulmonary arterial hypertension. *Eur Respir J*. 2009;33(2):338-44.
3. Pugh ME, Robbins IM, Rice TW, West J, Newman JH, Hennes AR. Unrecognized glucose intolerance is common in pulmonary arterial hypertension. *J Heart Lung Transplant*. 2011;30(8):904-11.
4. Lopez-Lopez JG, Moral-Sanz J, Frazzi Ziano G, Gomez-Villalobos MJ, Flores-Hernandez J, Monjaraz E, et al. Diabetes induces pulmonary artery endothelial dysfunction by NADPH oxidase induction. *Am Physiol Lung Cell Mol Physiol*. 2008;295(5):727-32.
5. Moral-Sanz J, Menendez C, Moreno L, Moreno E, Cogolludo A, Perez-Vizcarra E. Pulmonary arterial dysfunction in insulin resistant obese Zucker rats. *Respir Res*. 2012;13(5).
6. American Diabetes A. Diagnosis and classification of diabetes mellitus. *Diabetes Care*. 2008;31 Suppl 1:S55-60.
7. Alegria E, Cordero A, Llacuna M, Grim A, Leon M, Casanova JA, et al. Prevalence of Metabolic Syndrome in the Spanish Working Population: MESYAS Registry. *Rev Esp Cardiol (Engl Ed)*. 2005;58(7):797-806.
8. Hadareh F, Khalili D, Ghasemi A, Tohidi M, Sheikholeslami F, Azizi F. Triglyceride/HDL-cholesterol ratio is an independent predictor for coronary heart disease in a population of Iranian men. *Nutr Metab Cardiovasc Dis*. 2009;19(6):401-8.
9. Murguia-Romero M, Jimenez-Flores JR, Sigrist-Flores SC, Espinoza-Camacho MA, Jimenez-Morales M, Pina E, et al. Plasma triglyceride/HDL-cholesterol ratio is insulin resistant, and cardiometabolic risk in young adults. *J Lipid Res*. 2013;54(10):2795-9.
10. Guyatt GH, Sullivan MJ, Thompson PJ, Fallen EL, Pugsley SO, Taylor DW, et al. The 6-minute walk: a new measure of exercise capacity in patients with chronic heart failure. *Can Med Assoc J*. 1985;132(8):399-23.
11. Mostafá SA, Davies MJ, Morris DH, Yates T, Sriniwasan BT, Webb D, et al. The association of the triglyceride-to-HDL cholesterol ratio with insulin resistance in White European and South Asian men and women. *PloS One*. 2012;7(12):e50931.
12. Salazar MR, Carbajal HA, Espeche GC, Leiva Simniguez CE, March CE, Balbin E, et al. Comparison of the abilities of the plasma triglyceride/high-density lipoprotein cholesterol ratio and the metabolic syndrome to identify insulin resistance. *Diab Vasc Dis Res*. 2013;30(4):346-52.
13. Movahed MR, Hashemzadeh M, Jamal MM. The prevalence of pulmonary embolism and pulmonary hypertension in patients with type II diabetes mellitus. *Chest*. 2005;128(5):3568-71.
14. Esteghamati A, Gouya MM, Abbasi M, Delavari A, Alikhani S, Alaedinif F, et al. Prevalence of diabetes and impaired fasting glucose in the adult population of Iran: National Survey of Risk Factors for Non-Communicable Diseases of Iran. *Diabetes Care*. 2008;31(1):96-8.
15. Yan Z, Liu Y, Huang H. Association of glycated hemoglobin level with lipid ratio and individual lipids in type 2 diabetic patients. *Asian Pac J Trop Med*. 2012;5(6):469-71.
16. Niranjan G, Arun MS, Sriniwasan AR, Muthurangan G, Saha S, Ramasamy R. Association of Levels of Hba1c with Triglyceride/HDL-cholesterol ratio, insulin resistance, and cardiometabolic risk in young adults. *Diabetes Care*. 2005;28(5):1358-61.
17. Hansmann G, Wagner RA, Schellong S, Perez VA, Urashima T, Wang L, et al. Pulmonary arterial hypertension is linked to insulin resistance and reversed by peroxisome proliferator-activated receptor-gamma activation. *Circulation*. 2007;115(10):1275-84.
18. West J, Niewoehner KD, Johnson JA, Pugh ME, Gleaves L, Fessel JP, et al. A potential role for insulin resistance in experimental pulmonary hypertension. *Eur Respir J*. 2013;41(4):1386-91.
19. Mereles D, Ehlken N, Brescher S, Ghofrani S, Hoeper MM, Halank M, et al. Exercise and respiratory training improve exercise capacity and quality of life in patients with severe chronic pulmonary hypertension. *Circulation*. 2009;120(4):1482-9.