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Insulin resistance in pulmonary arterial hypertension

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ABSTRACT: Although obesity, dyslipidemia and insulin resistance (IR) are well known risk factors for systemic cardiovascular disease, their impact on pulmonary arterial hypertension (PAH) is unknown. The present authors' previous studies indicate that IR may be a risk factor for PAH. The current study has investigated the prevalence of IR in PAH and explored its relationship with disease severity.

Clinical data and fasting blood samples were evaluated in 81 nondiabetic PAH females. In total, 967 National Health and Nutrition Examination Surveys (NHANES) females served as controls. The fasting triglyceride to high-density lipoprotein cholesterol ratio was used as a surrogate of insulin sensitivity.

While body mass index was similar in NHANES *versus* PAH females (28.6 *versus* 28.7 kg·m^{-2}), PAH females were more likely to have IR (45.7 *versus* 21.5%) and less likely to be insulin sensitive (IS; 43.2 *versus* 57.8%). PAH females mostly (82.7%) had New York Heart Association (NYHA) class II and III symptoms. Aetiology, NYHA class, 6-min walk-distance and haemodynamics did not differ between IR and IS PAH groups. However, the presence of IR and a higher NYHA class was associated with poorer 6-months event-free survival (58 *versus* 79%).

Insulin resistance appears to be more common in pulmonary arterial hypertension females than in the general population, and may be a novel risk factor or disease modifier that might impact on survival.

KEYWORDS: Insulin resistance, obesity, pulmonary arterial hypertension

Pulmonary arterial hypertension (PAH) is characterised by progressive obliteration of pulmonary arterioles leading to increased pulmonary vascular resistance, right-heart failure and death. While recent USA epidemiological data report an increase in hospitalisations and mortality from PAH due to increased physician awareness and better diagnostic approaches [1], no treatment has been shown to be universally effective or curative. The pathobiology of PAH is complex and multifactorial. Therefore, it is unlikely that only one factor, pathway or gene mutation will explain all forms and cases [2]. This underscores the importance of continued efforts to explore other pathways and potential environmental modifiers of PAH.

Although obesity, dyslipidemia, and insulin resistance (IR) are well studied risk factors for systemic cardiovascular disease [3–5], their impact on PAH is unknown. Several clinical and laboratory observations suggest a link between IR and PAH. Obesity has been associated with IR in nondiabetic, normotensive subjects [6–8]. A recent study suggests that obesity in and of itself (aside from its link to appetite suppressant use) may be an overlooked risk factor for PAH [9]. Obesity appears to be common in PAH patients [10–13] and when coupled with a lack of physical activity (as in a deconditioned state) may predispose these patients to the development of IR [6, 14]. IR has also been linked to congestive heart failure (CHF) and idiopathic cardiomyopathy [15–17], conditions that may share pathophysiological profiles (such as myocardial strain) with PAH. Furthermore, elevation of inflammatory cytokines and other factors that lead to IR [18] have also been implicated in the pathogenesis of PAH. These include interleukin (IL)-6 [19, 20], monocyte chemotactic protein (MCP)-1 [21], endothelin (ET)-1 [22–24], and the endogenous nitric oxide synthase inhibitor, asymmetric dimethylarginine

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[25, 26]. Finally, the present authors have recently shown in a novel animal model that IR increases the susceptibility to PAH [27]: apolipoprotein E deficient (apoE$^{-/-}$) mice that became IR on a high fat diet did not upregulate the insulin sensitisers, adiponectin and leptin, and developed PAH, right ventricular hypertrophy and pulmonary vascular remodelling.

Based on the current authors' clinical observations, suggestive literature and laboratory results it was hypothesised that IR is: 1) more common in PAH patients and; 2) may be associated with severity of disease. In the present study, a cohort of PAH patients was stratified by an IR profile and compared with a matched control population using The National Health and Nutrition Examination Surveys (NHANES). The current analysis focused on females since PAH is a female predominant disease. For the first time it has been shown that the prevalence of IR is higher in female PAH patients than in the general population, and may be a novel risk factor or disease modifier.

METHODS

Study design and population

Using a case–control design, data from the NHANES 2003–2004 were evaluated for the prevalence of IR in a nondiabetic population and compared with a female PAH cohort. Subjects were excluded if they had a known history of diabetes mellitus, a fasting blood glucose of >126 mg·dL^{-1}, a haemoglobin A1C of >7.0, or pulmonary capillary wedge pressure of >15 mmHg (1.99 kPa). Lipid panel testing from 81 patients with PAH was undertaken during clinic visits or cardiac catheterisation at Stanford University Medical Center Adult Pulmonary Hypertension Clinic (Stanford, CA, USA). Detailed demographic, functional, haemodynamic and other data were obtained at the initial and subsequent clinic visits and entered into a relational database. Data were collected and analysed in accordance with institutional review board guidelines.

Definitions

The triglyceride (TG)/high-density lipoprotein cholesterol (HDL-C) ratio was used as a surrogate measure of IR profile. TG/HDL-C has been shown to be as sensitive and specific as fasting insulin in determining IR in both obese nondiabetic individuals [28, 29] and in females with polycystic ovarian syndrome [30]. Based on these studies, an individual was defined as IR when the TG/HDL-C ratio was >3.0, and insulin sensitive (IS) when TG/HDL-C ratio was <2.0. Subjects with a body mass index (BMI) of $\geqslant$25 kg·m^{-2} were considered as overweight and those with a BMI $\geqslant$30 kg·m^{-2} as obese.

Statistical analysis

The Kolmogorov–Smirnov test was applied to all data to test for normal distribution. An unpaired, two tailed t-test and a Chi-squared analysis were used for comparison between the two groups. The nonparametric Mann–Whitney U-test was used when data were not normally distributed. Spearman's rank test was used for determining correlation coefficients and univariate Cox regression analysis was used to calculate hazard ratios. The 6-month event-free survival (defined by death, transplantation, or hospitalisation for PAH exacerbation or right-heart failure) was estimated using the Kaplan–Meier method and analysed *via* the log-rank test. Demographic and clinical data are reported as mean±SD. Laboratory data (TG, HDL and the TG/HDL-C ratio) are reported as mean±SEM. A p-value of <0.05 was considered statistically significant.

RESULTS

Population characteristics

In the 967 female control subjects (NHANES), mean age was 49.1±19.3 yrs and BMI was 28.6±6.7 kg·m^{-2}. The female PAH cohort had a mean age of 46.1±11.4 yrs and a mean BMI of 28.7±7.5 kg·m^{-2}. There was no significant difference between age (p>0.05) and BMI (p>0.05) between NHANES and PAH female subjects. The racial/ethnicity profile of both female groups were similar (table 1).

Despite the demographic similarities between the NHANES and PAH cohorts, the prevalence of IR and metabolic profiles were significantly different (table 1). The prevalence of IR was significantly higher in the PAH females than in the well-matched NHANES controls (45.7 *versus* 21.5%; Chi-squared=24.2; degrees of freedom (df) 2; p<0.0001). Conversely, the majority of NHANES females (n=559; 57.8%), but less than half (n=35; 43.2%) of female PAH patients were IS. The mean TG/HDL-C ratio for the entire PAH female cohort identified patients as being overall IR, and was significantly higher (3.02±0.24 *versus* 2.3±0.09; p<0.001) than the NHANES controls (table 2).

IR NHANES females were older (51.7±18.9 *versus* 47.8±19.4 yrs; p<0.01), had a higher BMI (31.1±6.4 *versus* 27.3±6.5 kg·m^{-2}; p<0.0001) and a higher blood pressure (127.7±23 *versus* 121.1±22.3 mmHg; p<0.0001) than IS NHANES females (table 2). In contrast, IR PAH females were neither older nor more overweight/obese than their IS counterparts and had similar systemic blood pressures (table 2). Interestingly, IR PAH females were younger (45±11.0 *versus*

TABLE 1 Female demographic and metabolic characteristics

Parameter	NHANES	PAH	p-value#
Subjects n	967	81	
Age yrs	49.1±19.3	46.1±11.4	>0.05
BMI kg·m^{-2}	28.6±6.7	28.7±7.5	>0.05
Race/ethnicity			
White	535 (55.3)	54 (66.7)	
Hispanic	198 (20.5)	13 (16.0)	>0.05
Other	234 (24.2)	14 (17.3)	
Insulin sensitive	559 (57.8)	35 (43.2)	
Insulin resistant	208 (21.5)	37 (45.7)	<0.0001
Indeterminate	200 (20.7)	9 (11.1)	
Cardiovascular disease	58 (6.0)		

Data are presented as mean±SD and n (%), unless otherwise stated. NHANES: The National Health and Nutrition Examination Survey; PAH: pulmonary arterial hypertension; BMI: body mass index. Data collection was in nondiabetic subjects in the NHANES 2003–2004 cohort and PAH 2003–2006 cohort. Triglyceride (TG)/high-density lipoprotein cholesterol (HDL-C) ratio characterises individuals as insulin sensitive (TG/HDL-C<2) or insulin resistant (TG/HDL-C>3). #: p-values for age and BMI were based on Mann–Whitney U-test and Chi-squared analysis for race/ethnicity and insulin resistance profile.

TABLE 2 Demographic and metabolic profiles of insulin sensitive (IS) and insulin resistant (IR) females

Parameter	NHANES			PAH			p-values#		
	Total	IS	IR	Total	IS	IR	Total	IS	IR
Subjects n	967	559	208	81	35	37			
Age yrs	49.1±19.3	47.8±19.4	51.7±18.9¶	46.1±11.4	46.7±11.8	45±11.0	>0.05	>0.05	<0.05
BMI kg·m^{-2}	28.6±6.7	27.3±6.5	31.1±6.4+	28.7±7.5	29.2±8.8	28±6.3	>0.05	>0.05	<0.01
Blood pressure mmHg									
Systolic	123.6±23.1	121.1±22.3	127.7±23+	111.3±18.8	110.9±17.3	109.2±18.9	<0.0001	<0.05	<0.0001
Diastolic	68.8±11.7	68.4±11.1	70.4±13.2¶	69.1±11.4	69.5±13.6	66.8±7.5	>0.05	>0.05	>0.05
TG mg·dL^{-1}	121.1±3.26	77.7±1.18	227.3±11.70+	113.8±6.38	73.9±4.15	152.6±9.9+	>0.05	>0.05	<0.0001
HDL-C mg·dL^{-1}	60.5±0.54	67.7±0.68	46.7±0.75+	43.3±1.58	51.4±1.88	35.1±2.14+	<0.0001	<0.0001	<0.0001
TG/HDL-C ratio	2.30±0.09	1.19±0.02	5.16±0.36+	3.02±0.24	1.44±0.06	4.67±0.38+	<0.0001	<0.05	>0.05
CVD									
Yes	58 (6)	27 (4)	20 (9.6)						
No	905 (93.6)	530 (94.8)	187 (89.9)						
Unknown	4 (0.4)	2 (0.4)	1 (0.5)						

Data are presented as n (%) and mean±SEM, unless otherwise stated. Age, body mass index (BMI) and blood pressure are presented as mean±SD. NHANES: The National Health and Nutrition Examination Survey; PAH: pulmonary arterial hypertension; TG: triglyceride; HDL-C: high-density lipoprotein cholesterol; CVD: cardiovascular disease. Data were collected from nondiabetic female subjects. The TG/HDL-C ratio characterises individuals as IS (TG/HDL-C<2) or IR (TG/HDL-C>3). #: p-values are based on the Mann–Whitney U-test for comparison between NHANES *versus* PAH cohorts. For comparison of IR *versus* IS within each cohort: ¶: p<0.01; +: p<0.0001.

51.7±18.9 yrs; p<0.05) and less overweight than IR NHANES controls (BMI 28±6.3 *versus* 31.1±6.4 kg·m^{-2}; p<0.01), suggesting that IR may be a PAH risk factor independent of age and obesity.

IR was further characterised by higher TGs (NHANES 227.3±11.7 *versus* 77.7±1.18 mg·dL^{-1}; PAH 152.6±9.9 *versus* 73.9±4.15 mg·dL^{-1}; p<0.0001), and lower HDL-C (NHANES 46.7±0.75 *versus* 67.7 ± 0.68 mg·dL^{-1}; PAH 35.1±2.14 *versus* 51.4±1.88 mg·dL^{-1}; p<0.0001) in both NHANES and PAH cohorts (table 2). While TG/HDL-C ratio was higher in the IS PAH females than in the IS NHANES females (1.44±0.07 *versus* 1.19±0.02; p<0.05), there was no significant difference in TG/HDL-C ratio between the IR groups (4.67±0.38 *versus* 5.16±0.36; p>0.05). However, IR PAH females had significantly lower HDL-C levels than IR NHANES controls (35.1±2.14 *versus* 46.7±0.75 mg·dL^{-1}; p<0.0001).

PAH disease characteristics

The majority (82.7%) of female PAH patients were either New York Health Association (NYHA) class II and III (table 3). Using the World Health Organization group classification, the cohort consisted of 34.6% idiopathic PAH patients, 19.8% stimulant and/or anorexigen associated PAH patients, 32.1% collagen vascular disease (CVD) patients, 7.4% congenital heart disease patients, 4.9% portopulmonary hypertension patient and 1.2% HIV associated PAH patients. While the majority of the PAH cohort was receiving mono or dual disease-specific therapies, 34.6% were not on treatment at the time of evaluation. Haemodynamic data indicated a severe but compensated group as judged by a mean pulmonary artery pressure of 53.7±12.8 mmHg, cardiac index of 2.25±0.6 L·min^{-1}·m^{-2} and pulmonary vascular resistance of 12.3±5.4 WU (n=74). While patients with NYHA class III and IV symptoms had a lower 6-min walk distance (6MWD; 334±151 *versus* 472.8±109.5 m; p<0.0001) and a trend towards a higher TG/HDL-C ratio (3.24±0.4 *versus* 2.68±0.22) compared with those with class I and II symptoms, the difference in the TG/HDL-C ratio was not statistically significant (see online supplementary material table S1). Moreover, the TG/HDL-C ratio itself did not correlate with NYHA class, 6MWD or haemodynamics (see online supplementary material table S2).

There were no differences in age, BMI, NYHA classification (Chi-squared=2; df 2; p>0.05), baseline arterial oxygen saturation measured by pulse oximetry, use of hormone replacement therapies, or systemic blood pressure between the IS and IR female PAH groups (tables 2 and 3 and online supplementary material table S1). There was also no significant difference in the 6MWD between the IR and IS groups (390±137 *versus* 417±172 m). Although there was no difference in the distribution of the number of PAH specific therapies instituted (Chi-squared=4.5; df 2; p>0.05), more PAH patients with IR were on prostanoid therapy (17 (46%) out of 37) than their IS counterparts (10 (28.6%) out of 35). Baseline haemodynamics were similar between IS and IR groups.

Despite the similar clinical profiles of the two cohorts, the IR group had a significantly worse 6-month event-free survival (fig. 1, table 4) compared with their IS counterparts (58% IR *versus* 79% IS; p<0.05). The combined risk of hospitalisation for right-heart failure, transplantation or death (when adjusted for age and BMI) was strongly associated with an advanced NYHA class (hazard ratio (HR) 3.79, 95% confidence interval (CI) 1.75–8.22; p<0.01) and IR (HR 2.57, 95% CI 1.03–6.06; p<0.05), but not with ET receptor antagonist therapy (HR 0.81, 95% CI 0.31–2.14;

TABLE 3 Comparison of female pulmonary arterial disease (PAH) patients' disease characteristics based on metabolic profiles

	Total	IS	IR	p-value
Subjects n	81	37	37	
Aetiology				
IPAH	28 (34.6)	14 (40.0)	12 (32.4)	
Stimulant and anorexigen	16 (19.8)	8 (22.8)	5 (13.5)	
CHD	6 (7.4)	0	5 (13.5)	>0.05
CVD	26 (32.1)	10 (28.6)	13 (35.2)	
PPHTN	4 (4.9)	3 (8.6)	1 (2.7)	
HIV	1 (1.2)	0	1 (2.7)	
6MWD m	404±146	417±172	390±137	>0.05
Oxygen saturation %	95	94	95	>0.05
NYHA				
I	2 (2.5)	1 (2.9)	1 (2.7)	
II	31 (38.3)	13 (37.1)	15 (40.5)	
III	36 (44.4)	17 (48.5)	13 (35.2)	>0.05
IV	10 (12.3)	3 (8.6)	7 (18.9)	
Unknown	2 (2.5)	1 (2.9)	1 (2.7)	
Therapies#				
None	28 (34.6)	14 (40.0)	11 (29.7)	
Monotherapy	37 (45.7)	12 (34.3)	21 (56.8)	>0.05
Dual therapy (or more)	16 (19.7)	9 (25.7)	5 (13.5)	
Prostanoid	30 (37.0)	10 (28.6)	17 (46)	
ETA	28 (34.6)	12 (34.3)	11 (29.7)	
PDE-I	14 (17.3)	8 (22.8)	3 (8.1)	
HRT	3 (3.7)	2 (5.7)	1 (2.7)	>0.05
Blood pressure mmHg				
Systolic	111.3±18.8	110.9±17.3	109.2±18.9	>0.05
Diastolic	69.1±11.4	69.5±13.6	66.8±7.5	>0.05
Haemodynamics				
MRA mmHg	9.5±5	9.4±5.1	10.2±5.7	>0.05
$\bar{P}_{pa}$ mmHg	53.4±12.8	51.7±13.6	54.2±12.6	>0.05
CI L/min/m^2	2.24±0.6	2.28±0.6	2.21±0.7	>0.05
PVR WU	12.3±5.4	12.0±5.5	12.3±5.6	>0.05

Data are presented as mean±SD or n (%), unless otherwise stated. IS: insulin sensitive; IR: insulin resistant; IPAH: idiopathic PAH; CHD: congenital heart disease; CVD: collagen vascular disease; PPHTN: portopulmonary hypertension; 6MWD: 6-min walk distance; NYHA: New York Heart Association; ETA: endothelin antagonist; PDE-I: phosphodiesterase inhibitors; HRT: hormone replacement therapies; MRA: mean right-arterial pressure; $\bar{P}_{pa}$: mean pulmonary artery pressure; CI: cardiac index; PVR: pulmonary vascular resistance. #: therapies include prostanoid, epoprostenol, iloprost, or treprostinil and ETA and PDE-I.

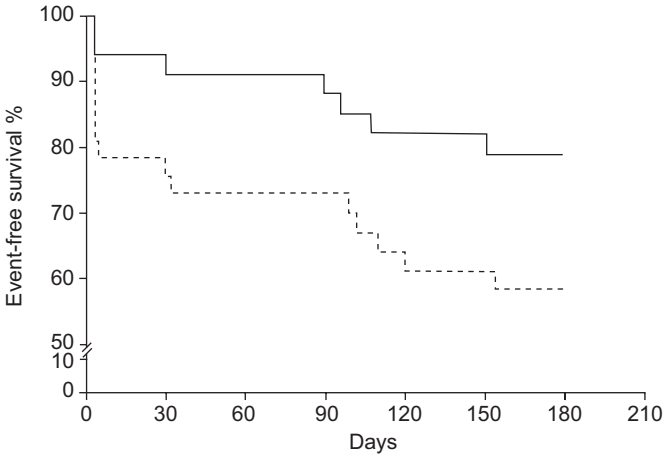

FIGURE 1. Kaplan–Meier 6-month event-free survival curve in pulmonary arterial hypertension (PAH) females. Insulin sensitive (———) PAH females had significantly better outcome compared with their insulin resistant (– – – – –) counterparts (79 *versus* 58%; p<0.05). Events were defined as death, transplantation, or acute hospitalisation due to PAH exacerbation or right-heart failure.

p>0.05), prostanoid therapy (HR 1.24, 95% CI 0.47–3.11; p>0.05), 6MWD (HR 0.99, 95% CI 0.98–0.99; p>0.05), or cardiac index (HR 0.54, 95% CI 0.24–1.26; p>0.05).

DISCUSSION

The past 20 yrs have seen a remarkable increase in the number of children, adolescents [4] and adults [5] with the metabolic syndrome at high-risk for systemic CVD. However, it was not known whether the metabolic syndrome, and especially its key element, IR, is associated with clinical PAH. In the present study, it was shown for the first time that IR is more prevalent in female patients with PAH than in the general female population. While the prevalence of IR in the NHANES female population was influenced by age and degree of obesity, these factors did not account for the increased prevalence of IR in the PAH cohort. Although IR is more common in the obese control subjects and PAH females, the current data suggest that obesity alone does not account for the higher prevalence of IR in PAH females.

Surprisingly, a significant difference in PAH aetiology, NYHA functional classification, number of disease specific therapies or haemodynamics was not found between IR and IS PAH groups. While IR is associated with a more advanced NYHA class and reduced 6MWD in patients with CHF [31–33], the present PAH cohort did not exhibit this relationship. However, similar to patients with CHF, IR in the current study was associated with poorer survival (fig. 1, table 4) and was a strong predictor of acute hospitalisation for right-heart failure, transplantation or death. Indeed, an advanced NYHA functional class and the presence of IR was shown in the present PAH cohort to be associated with worse event-free survival. The current findings suggest that IR is not solely a result of severity of illness in females with PAH, but potentially a new risk factor for disease progression and worse outcomes.

IR in PAH may be a disease modifier rather than simply a metabolic epiphenomena. Such a hypothesis is further fuelled by the fact that several key PAH associated conditions (connective tissue diseases, HIV and stimulant use) have also been linked to IR [34–38]. These associated conditions are linked to IR by an underlying inflammatory pathology, a common theme in PAH. In accordance with these observations, many pro-inflammatory cytokines, such as IL-6 [19, 20] and MCP-1 [21] (also known as chemokine C-C motif ligand [39]), are elevated in IR and PAH. The milieu of IR may provide an increased susceptibility for development or

TABLE 4 Event-free survival in pulmonary arterial hypertension females

Days	Insulin sensitive n	Insulin resistant n
0	35	37
30	32	28
60	31	26
90	30	26
120	28	22
150	27	22
180	26	20

accelerated progression of PAH in the presence of detrimental conditions (connective tissue disease, congenital heart disease and environmental exposures) or genetic mutations (*e.g.* bone morphogenetic protein receptor II, serotonin transporter and potassium-ion channels).

Assuming that IR contributes to the pathophysiology of PAH, it is reasonable to suggest that interventions that enhance insulin sensitivity in patients with PAH could be of clinical benefit. Since both excess adiposity and sedentary behaviour adversely affect insulin action, an obvious choice would be weight loss and increased physical activity, interventions that have been accomplished safely in PAH patients [40]. PAH patients who underwent a 15-week exercise programme had a significant improvement in 6MWD (91 ± 61 m) when compared with control PAH patients (-15 ± 54 m) [40]. Although many factors could explain the benefits of exercise in these PAH patients, and many mechanisms could be invoked to explain an improved 6MWD, it is plausible that such enhancement is associated with improved insulin and lipid profiles. Interestingly, physical training has also been shown to improve hyperinsulinaemia and IR in patients with CHF [41]. The mechanism of development of IR is extremely complex and may be influenced by hypoxaemia and a deconditioned state. The present authors did not find any differences in baseline hypoxaemia to account for the increased prevalence of IR, but the current study was not designed to determine the impact of exercise-induced desaturation or deconditioning on IR in PAH patients.

Beyond diet and exercise, current and future pharmacotherapy for PAH may target the pathways directly or indirectly involved in IR. It has been suggested that ET-1 antagonists may exert some of their effects on the pulmonary vasculature *via* insulin sensitising pathways [42, 43]. Peroxisome proliferator-activated receptor (PPAR)γ agonists of the thiazolidendiones class are commonly used in the treatment of diabetes, increased insulin sensitivity, lower circulating plasma insulin levels [44, 45], and improved vascular abnormalities [46, 47] in IR individuals. Recently, the current authors have shown that PAH is linked to IR in apoE$^{-/-}$ mice on a high fat diet. Intriguingly, a 4-week treatment with a PPARγ agonist led to an eight-fold increase in plasma adiponectin, improved insulin sensitivity, and complete regression of PAH, right ventricular hypertrophy and abnormal peripheral pulmonary arterial muscularisation in IR apoE$^{-/-}$ mice. In accordance with these findings, it could be demonstrated that mice with targeted deletion of PPARγ in vascular smooth muscle cells develop PAH, right ventricular hypertrophy and pulmonary vascular remodeling in room air [48]. Hence, PPARγ agonists may play an important role in the future treatment of PAH patients.

While the present findings are stimulating, there are limitations to this study. Markers of IR that have been studied in the general population or in patients with systemic CVD still need to be validated in patients with PAH. There are currently no studies evaluating the utility of fasting insulin, fasting glucose, the homeostasis model assessment, or the quantitative insulin sensitivity check index as markers of IR in PAH. The current authors' choice to use TG/HDL-C as a surrogate of IR in PAH was based on published evidence [28–30] and recognition of its reliability (see online supplementary material figs S1 and S2). Furthermore, these findings may have been confounded by multiple drug therapies with different efficacies, drug–drug and drug–hormone interactions. Screening a larger cohort of untreated PAH patients and following their insulin profiles over time (while initiated and continued on therapy) may result in more comprehensive insights into the exact role of IR in PAH. Finally, future studies should attempt to delineate the impact of sex on the development of IR in PAH, an issue that the current study could not fully address. A limited analysis of a cohort of 27 male PAH patients did not reveal an increased prevalence of IR (see online supplementary material table S3).

In conclusion, insulin resistance is more prevalent in females with pulmonary arterial hypertension than in the general population and may be a novel risk factor or disease modifier associated with poorer outcome. While the aetiology of pulmonary arterial hypertension is likely to be multifactorial, the present authors suggest that insulin resistance may represent an important risk factor to disease development and/or its progression. If the present findings hold true in a substantial proportion of pulmonary arterial hypertension patients, then treatment aimed at improving insulin resistance, *via* simple measures such as diet and exercise, or new pharmacologic approaches, may benefit a large percentage of patients with pulmonary arterial hypertension.

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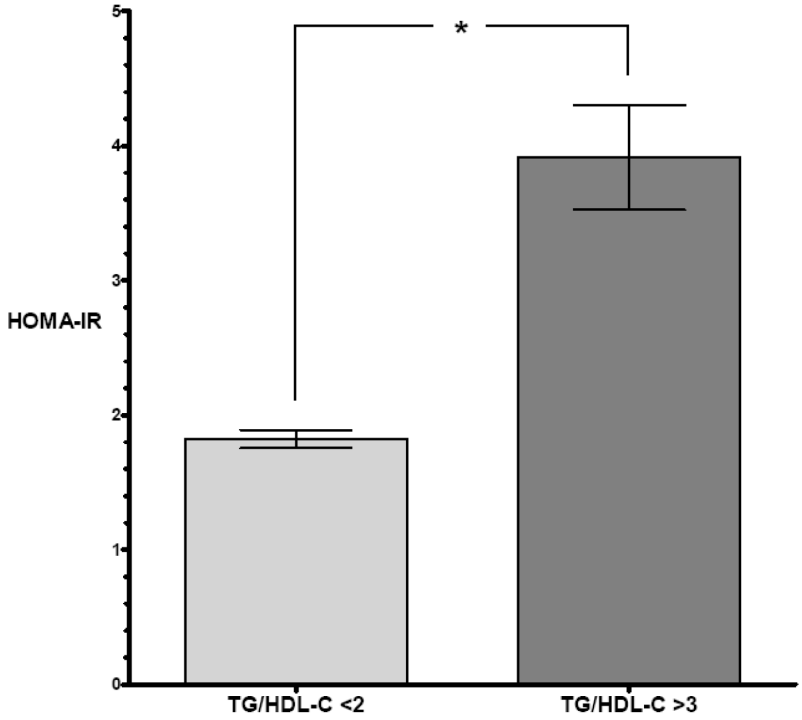

FIGURE S1. Determination of Insulin Resistance by TG/HDL-C is validated by HOMA-IR calculation in NHANES females. Assessment of insulin resistance by the homeostasis model (HOMA-IR) shows a significantly lower value (* $p<0.0001$) in subjects deemed insulin sensitive by TG/HDL-C ratio <2 than those with TG/HDL-C > 3. HOMA-IR for TG/HDL<2=1.82±0.067 (n=559) and for TG/HDL>3=3.91±0.39 (n=208); values indicate Mean±SEM. HOMA-IR = fasting glucose (mmol/l) x fasting insulin [microU/ml]/22.5). TG/HDL-C = Triglyceride (TG) to high-density lipoprotein cholesterol (HDL-C) ratio.

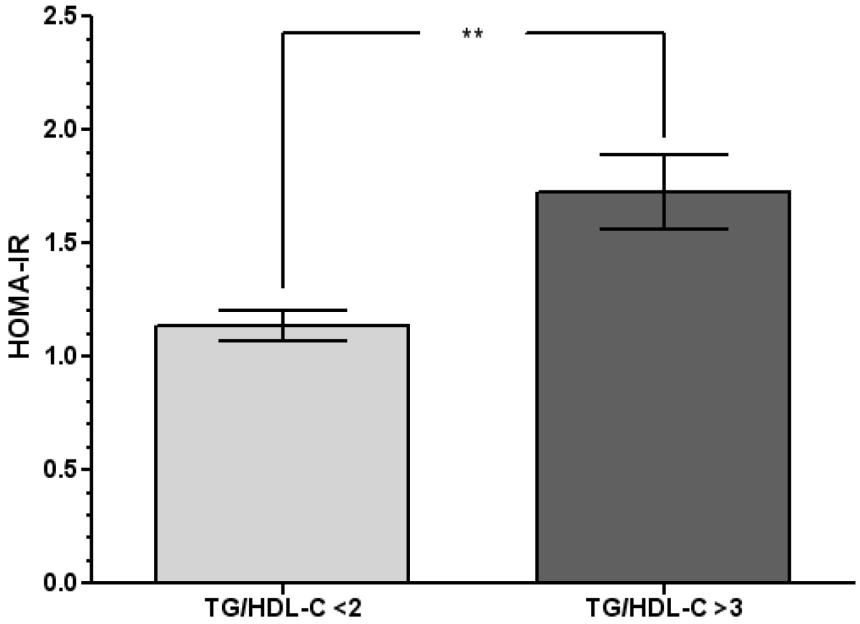

FIGURE S2. Determination of Insulin Resistance by TG/HDL-C is validated by HOMA-IR calculation in NHANES females with BMI<25 kg/m^2. Assessment of insulin resistance by the homeostasis model (HOMA-IR) shows a significantly lower value (** p<0.0001) in non-obese/non-overweight subjects deemed insulin sensitive by TG/HDL-C ratio <2 than those with TG/HDL-C > 3. HOMA-IR for TG/HDL<2=1.14±0.07 (n=218) and for TG/HDL>3=1.73±0.16 (n=40); values indicate Mean±SEM. HOMA-IR = fasting glucose (mmol/l) x fasting insulin [microU/ml]/22.5). TG/HDL-C = Triglyceride (TG) to high-density lipoprotein cholesterol (HDL-C) ratio.

Parameter	NYHA I&II (n=33)	NYHA III&IV (n=46)	p-value
Age - yrs	45.2±12.2	46.9±11.1	>0.05
BMI - kg/m^2	27.9±7.8	29.3±7.4	>0.05
6MWD - m	472.8±109.5	334±151	<0.0001
Metabolic Profile:			
Mean TG/HDL-C Ratio	2.68±0.22	3.24±0.4	>0.05
Insulin Sensitive - n (%)	15 (45.5)	17 (37)	
Insulin Resistant - n (%)	15 (45.5)	23 (50)	>0.05
Indeterminate - n (%)	3 (9)	6 (13)	

Table S1. Characterization of metabolic profile by functional class in PAH females. PAH females with a more advanced functional class (NYHA III & IV) have a significantly lower 6MWD ($p<0.0001$) than those with NYHA I & II symptoms. While patients with more advanced functional class appear be more insulin resistant (TG/HDL-C NYHA III & IV = 3.24±0.4 vs NYHA I & II = 2.68±0.22), the difference is not statistically significant ($p>0.05$). Moreover, the prevalence of insulin resistance is not different between the 2 groups. NYHA = New York Heart Association Functional Class, BMI = Body Mass Index, 6MWD = 6-Minute Walk Distance, TG/HDL-C = Triglyceride (TG) to high-density lipoprotein cholesterol (HDL-C) ratio. All values reported as Mean±SD.

	TG/HDL-C Ratio	
Parameter	Spearman's r	p-value
NYHA class	0.09	>0.05
6MWD	-0.1	>0.05
SpO_2	0.04	>0.05
mRA	0.11	>0.05
mPAP	0.12	>0.05
Ci	-0.16	>0.05
PVR	0.07	>0.05

Table S2. Correlations between TG/HDL-C ratio and clinical disease parameters in females with PAH. Selected parameters such as NYHA class, 6MWD, baseline pulse oximetry (SpO2), and hemodynamics are not directly correlated with the TG/HDL-C ratio. PAH = Pulmonary Arterial Hypertension, NYHA = New York Heart Association Functional Class, 6MWD = 6-Minute Walk Distance, mRA = mean right atrial pressure, mPAP = mean pulmonary artery pressure, Ci = cardiac index, PVR = pulmonary vascular resistance, and TG/HDL-C = Triglyceride (TG) to high-density lipoprotein cholesterol (HDL-C) ratio.

Parameter	**NHANES** (n=891)	**PAH** (n=27)	p*
Age - yr	48.9±19.3	43.8±11.9	>0.05
BMI - kg/m^2	27.8±5.5	29.8±6.3	>0.05
Race/Ethnicity - n (%)			
White	486 (54.5)	18 (66.7)	0.01
Hispanic	206 (23.1)	5 (18.5)	
Other	199 (22.4)	4 (14.8)	
Insulin Sensitive - n (%)	378 (42.4)	11 (40.7)	>0.05
Insulin Resistant - n (%)	315 (35.4)	10 (37.1)	
Indeterminent - n (%)	198 (22.2)	6 (22.2)	
Cardiovascular Dz - n (%)	93 (10.4)	-	

Table S3. Demographic & Metabolic Characteristics of Male PAH Cohort. Analysis of a small cohort of PAH males does not demonstrate a higher prevalence of insulin resistance as compared with NHANES controls. Data collection was in nondiabetic male subjects: NHANES 2003-2004 cohort, PAH 2003-2006 cohort. All values indicate mean ±SD. Triglyceride (TG) to high-density lipoprotein cholesterol (HDL-C) ratio (TG/HDL-C) characterizes individuals as insulin sensitive (TG/HDL-C<2) or insulin resistant (TG/HDL-C>3). * p-values for Age & BMI were based on Mann-Whitney U test, and chi-squared analysis for Race/Ethnicity & insulin resistance profile. NHANES = National Health And Nutrition Surveys, BMI = Body Mass Index, PAH = Pulmonary Arterial Hypertension.