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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. Our previous studies indicate that IR may be a risk factor for PAH. We now investigate the prevalence of IR in PAH and explore its relationship to disease severity.

Clinical data and fasting blood samples were evaluated in 81 non-diabetic PAH females. National Health and Nutrition Examination Surveys (NHANES) females (n=967) served as controls. Fasting triglyceride to high-density lipoprotein cholesterol ratio (TG/HDL-C) was used as a surrogate of insulin sensitivity.

While BMI was similar in NHANES vs PAH females (28.6 vs. 28.7 kg/m^2), PAH females were more likely to be IR (45.7% vs. 21.5%) and less likely to be IS (43.2% vs. 57.8%, $p<0.0001$). PAH females mostly had NYHA class II and III symptoms (82.7%). Etiology, NYHA class, 6-minute-walk-distance, and hemodynamics did not differ between IR and IS PAH groups. However, the presence of IR and a higher NYHA class were associated with poorer 6-months event-free survival (58% vs. 79%, $p<0.05$).

Insulin Resistance appears to be more common in PAH females than in the general population, and may be a novel risk factor or disease modifier which might impact survival.

Keywords: Insulin Resistance, Obesity, Pulmonary Arterial Hypertension,

INTRODUCTION:

Pulmonary arterial hypertension (PAH) is characterized by progressive obliteration of pulmonary arterioles leading to increased pulmonary vascular resistance, right heart failure, and death. While recent US epidemiologic data report an increase in hospitalizations 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 *pulmonary* arterial hypertension is unknown. Several clinical and laboratory observations suggest a link between IR and PAH. Obesity has been associated with insulin resistance in non-diabetic, 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 lack of physical activity (as in a deconditioned state) may predispose these patients to the development of IR [6, 14]. Insulin resistance has also been linked to congestive heart failure (CHF) and idiopathic cardiomyopathy [15-17], conditions which may share pathophysiologic profiles (such as myocardial strain) with PAH. Furthermore, elevation of inflammatory cytokines and other factors that lead to insulin resistance [18] have also been implicated in the pathogenesis of PAH. These include interleukin 6 (IL-6) [19, 20], monocyte chemoattractant protein 1 (MCP-1) [21], endothelin-1 (ET-1) [22-24], and the endogenous nitric oxide synthase (NOS) inhibitor, asymmetric dimethylarginine (ADMA) [25, 26]. Finally, we have recently shown in a novel animal model that insulin resistance increases

the susceptibility to PAH [27]: Apolipoprotein E deficient (apoE -/-) mice that became insulin resistant on a high fat diet did not upregulate the insulin sensitizers adiponectin and leptin, and developed PAH, right ventricular hypertrophy, and pulmonary vascular remodeling.

Based on our clinical observations, suggestive literature, and laboratory results we hypothesized that insulin resistance is (1) more common in PAH patients, and (2) may be associated with severity of disease. In this study we stratified a cohort of PAH patients by insulin resistance profile and compared it to a matched control population using The National Health and Nutrition Examination Surveys (NHANES). Our analysis focused on women since PAH is a female predominant disease. We show for the first time that the prevalence of insulin resistance 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 National Health and Nutrition Examination Survey 2003-2004 (NHANES) were evaluated for the prevalence of insulin resistance in a non-diabetic population and compared to a female PAH cohort. Subjects were excluded if they had a known history of diabetes mellitus, a fasting blood glucose of greater than 126 mg/dl, a hemoglobin $A1_C$ ($HgA1_C$) of greater than 7.0, or pulmonary capillary wedge pressure of greater than 15 mmHg. Lipid panel testing from 81 patients with PAH was undertaken during clinic visits or cardiac catheterization at Stanford University Medical Center Adult Pulmonary Hypertension Clinic. Detailed demographic, functional, hemodynamic and other data were obtained at the initial and subsequent clinic visits and entered into a relational database. Data were gathered and analyzed in accordance with institutional review board guidelines.

Definitions. We used the Triglyceride (TG) to High-Density Lipoprotein (HDL-C) ratio (TG/HDL-C) as a surrogate measure of insulin resistance profile. TG/HDL-C has been shown to be as sensitive and specific as fasting insulin in determining insulin resistance in both obese non-diabetic individuals [28, 29] and in women with polycystic ovarian syndrome [30]. Based upon these studies, 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. We considered subjects with BMI$\geq$25 kg/m^2 as overweight and those with BMI$\geq$30 kg/m^2 as obese.

Statistical Analysis. The Kolmogorov-Smirnov test was applied to all data to test for normal distribution. Unpaired, two tailed t-test, and chi-squared analysis were used for comparison between two groups. We used the nonparametric Mann Whitney U test 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 six month event-free survival (defined by death, transplantation, or hospitalization for PAH exacerbation or right-heart failure) was estimated using the Kaplan-Meier method and analyzed via the log-rank test. Demographic and clinical data are reported as mean ± standard deviation (SD). Laboratory data (TG, HDL, and TG/HDL-C ratio) are reported as mean ± standard error of the mean (SEM). A p value of less than 0.05 was considered statistically significant.

RESULTS

Population Characteristics. In the 967 female control subjects (NHANES), mean age was 49.1±19.3 years and BMI was 28.6±6.7 kg/m^2. The female PAH cohort had a mean age of 46.1±11.4 years 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 insulin resistance and metabolic profiles were significantly different (**Table 2**). The prevalence of insulin resistance was significantly higher in the PAH females than in the well-matched NHANES controls (45.7% vs 21.5%, X^2=24.2, df 2, p<0.0001). Conversely, the majority of NHANES females (n=559, 57.8%), but less than half of female PAH patients (n=35, 43.2%) were insulin sensitive. Mean TG/HDL-C for the entire PAH female cohort identified them as being overall insulin resistant, and was significantly higher (3.02±0.24 vs 2.3±0.09, p<0.001) than the NHANES controls (**Table 2**).

Insulin resistant NHANES females were older (51.7±18.9 vs 47.8±19.4 years, p<0.01), had a higher BMI (31.1±6.4 vs 27.3±6.5 kg/m^2, p<0.0001), had higher blood pressure (127.7±23 vs 121.1±22.3 mmHg, p<0.0001) than IS NHANES females (**Table 2**). In contrast, insulin resistant PAH females were neither older nor more overweight/obese than their insulin sensitive counterparts and had similar systemic blood pressures (**Table 2**). Interestingly, insulin resistant PAH females were younger (45±11.0 vs 51.7±18.9 years, p<0.05) and less overweight than IR NHANES controls (BMI 28±6.3 vs 31.1±6.4 kg/m^2, p<0.01), suggesting that IR may be a PAH risk factor independent of age and obesity.

Insulin resistance was further characterized by higher triglycerides (NHANES: 227.3±11.7 vs. 77.7±1.18 mg/dL; PAH: 152.6±9.9 vs. 73.9±4.15 mg/dL, p<0.0001), and lower HDL-C (NHANES 46.7±0.75 vs. 67.7 ± 0.68 mg/dL; PAH: 35.1±2.14 vs. 51.4±1.88 mg/dL, p<0.0001) in both NHANES and PAH cohorts (**Table 2**). While mean TG/HDL-C ratio was higher in the IS PAH females than in the IS NHANES females (1.44±0.07 vs 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 vs 5.16±0.36, p>0.05). However, IR PAH females had significantly lower HDL cholesterol levels than IR NHANES controls (35.1±2.14 mg/dL vs. 46.7±0.75 mg/dL; p<0.0001).

PAH Disease Characteristics. The majority (82.7%) of female PAH patients were either New York Heart Functional class II and III (**Table 3**). By WHO group classification, the cohort consisted of 34.6% idiopathic PAH patients (IPAH), 19.8% stimulant and/or anorexigen associated PAH, 32.1% collagen vascular disease (CVD), 7.4% congenital heart disease (CHD), 4.9% portopulmonary hypertension (PPHTN), and 1.2% HIV associated PAH. While the majority of the PAH cohort was on mono or dual disease-specific therapies, 34.6% were not on treatment at the time of evaluation. Hemodynamic data indicated a severe but compensated group as judged by mean pulmonary artery pressure (mPAP) of 53.7±12.7 mmHg, cardiac index (CI) of 2.25±0.6 L/min/m^2, and pulmonary vascular resistance (PVR) of 12.3±5.4 WU (n=74). While patients with NYHA class III & IV symptoms had a lower 6MWD (334±151 vs 472.8±109.5 m, $p<0.0001$) and a trend towards higher mean TG/HDL-C ratio (3.24±0.4 vs 2.68±0.22) compared to those with class I & II symptoms, the difference in mean TG/HDL-C ratio was not statistically significant (**Online supplement Table S1**). Moreover, TG/HDL-C ratio itself did not correlate with NYHA class, 6MWD, or hemodynamics (**Online supplement, Table S2**).

There were no differences in age, BMI, NYHA classification ($X^2=2$, df 2, $p>0.05$), baseline pulse oximetry (SpO_2), use of hormone replacement therapies, or systemic blood pressure between the IS and IR female PAH groups (**Table 2, Table 3, and Online supplement Table S1)**. There was also no significant difference in the 6-minute walk distance (6MWD) between the IR and IS groups (390±137 vs 417±172 m). Though there was no difference in the distribution of number of PAH specific therapies instituted ($X^2=4.5$, df 2, $P>0.05$), more PAH patients with IR were on prostanoid therapy (17/37, 46%) than their IS counterparts (10/35, 28.6%). Baseline hemodynamics 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 (**Figure 1**) compared to their IS counterparts (58% IR vs 79% IS,

p<0.05). The combined risk of hospitalization for right heart failure, transplantation, or death (when adjusted for age and BMI) was strongly associated with an advanced NYHA class (hazard ratio 3.79, 95% confidence interval 1.75-8.22, p<0.01) and insulin resistance (HR 2.57, 95% CI 1.03-6.06, p<0.05) , but not with endothelin receptor antagonist therapy (HR 0.81, 95% CI 0.31-2.14, p>0.05), prostanoid therapy (HR 1.24, 95% CI 0.47- ,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 two decades have seen a remarkable increase in the number of children, adolescents [4] and adults [5] with the metabolic syndrome at high risk for *systemic* cardiovascular disease. However, it was not known whether the metabolic syndrome, and especially its key element, insulin resistance, is associated with clinical pulmonary arterial hypertension. In this study, we have shown for the first time that insulin resistance is more prevalent in female patients with pulmonary arterial hypertension than in the general female population. While the prevalence of insulin resistance 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 insulin resistance is more common in the obese control subjects *and* PAH females, our data suggest that obesity alone *does not* account for the higher prevalence of IR in PAH women.

Surprisingly, we did not find a significant difference in PAH etiology, NYHA functional classification, number of disease specific therapies, or hemodynamics between our IR and IS PAH groups. While insulin resistance is associated with more advanced NYHA class and reduced 6MWD in patients with congestive heart failure (CHF) [31-33], our PAH cohort does not exhibit this relationship. However, similar to patients with CHF, insulin resistance in our

study was associated with poorer survival (**Figure 1**) and a strong predictor of acute hospitalization for right heart failure, transplantation, or death. Indeed, we could show that an advanced NYHA functional class and presence of insulin resistance in our PAH cohort were associated with a worse age and BMI adjusted event-free survival. Our findings suggest that insulin resistance is not solely a result of severity of illness in women with PAH, but potentially a new risk factor for disease progression and worse outcomes.

Insulin resistance in PAH may be a disease modifier rather than simply a metabolic epiphenomena. Such hypothesis is further fuelled by the fact that several key PAH associated conditions (connective tissues diseases, HIV, and stimulant use) have also been linked to insulin resistance [34-38]. These associated conditions are linked to insulin resistance 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 CCL2 [39]), are elevated in insulin resistance and PAH. The milieu of insulin resistance may provide an increased susceptibility for development or accelerated progression of PAH in the presence of detrimental conditions (connective tissue disease, congenital heart disease, environmental exposures) or genetic mutations (e.g., bone morphogenetic protein receptor II, serotonin transporter, potassium-ion channels).

Assuming that insulin resistance contributes to the pathophysiology of PAH, it is reasonable to suggest that interventions which enhance insulin sensitivity in patients with PAH could be of clinical benefit. Since both excess adiposity and sedentary behavior adversely affect insulin action, an obvious choice would be weight loss and increased physical activity - interventions that have been accomplished safely PAH patients [40]: PAH patients who underwent a 15 week

exercise program had a significant improvement in mean 6MWD (91±61 m) when compared with control PAH patients (-15±54 m) [40]. Though 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 hyperinsulinemia and insulin resistance in patients with CHF [41]. The mechanism of development of insulin resistance is extremely complex and may be influenced by hypoxemia and a deconditioned state. We did not find any differences in baseline hypoxemia to account for increased prevalence of IR, but our study was not designed to determine the impact of exercise-induced desaturation or deconditioning on insulin resistance 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 endothelin-1 antagonists may exert some of their effects on the pulmonary vasculature via insulin sensitizing pathways [42, 43]. PPARγ agonists of the thiazolidendiones class are commonly used in the treatment of diabetes, increase insulin sensitivity, lower circulating plasma insulin levels [44, 45], and improve vascular abnormalities [46, 47] in insulin resistant individuals. Recently, we have shown in apoE deficient mice on high fat diet that PAH is linked to insulin resistance. Intriguingly, a 4-week treatment with a PPARγ agonist led to an 8-fold increase in plasma adiponectin, improved insulin sensitivity, and complete regression of PAH, right ventricular hypertrophy and abnormal peripheral pulmonary arterial muscularization in insulin-resistant apoE deficient mice. In accordance with these findings, we could demonstrate that mice with targeted deletion of PPARγ in vascular smooth muscle cells develop PAH, RVH and pulmonary vascular remodeling in room air [48] Hence, PPARγ agonists may play and important role in the future treatment of PAH patients.

While our findings are stimulating, there are limitations to this study. Markers of insulin resistance which have been studied in the general population or in patients with systemic cardiovascular disease 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 (HOMA), or the quantitative insulin sensitivity check index as markers of IR in PAH. Our choice to use TG/HDL-C as a surrogate of insulin resistance in PAH was based on published evidence [28-30] and recognition of its reliability (**Online supplement Figures S1 and S2**). Furthermore, our 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 insulin resistance in PAH. Finally, future studies should attempt to delineate the impact of gender on development of insulin resistance in PAH– an issue that our study could not fully address. A limited analysis of a cohort of male PAH patients (n=27) did not reveal an increased prevalence of IR (**Online supplement Table S3**).

In conclusion, insulin resistance is more prevalent in women with PAH than in the general population and may be a novel risk factor or disease modifier associated with poorer outcome. While the etiology of PAH is likely multifactorial, we suggest that insulin resistance may represent an important risk factor to disease development and/or its progression. If our findings hold true in a substantial proportion of PAH 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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REFERENCES

1. Hyduk A, Croft JB, Ayala C, Zheng K, Zheng ZJ, Mensah GA. Pulmonary hypertension surveillance--United States, 1980-2002. *MMWR Surveill Summ* 2005: 54(5): 1-28.

2. Humbert M, Morrell NW, Archer SL, Stenmark KR, MacLean MR, Lang IM, Christman BW, Weir EK, Eickelberg O, Voelkel NF, Rabinovitch M. Cellular and molecular pathobiology of pulmonary arterial hypertension. *Journal of the American College of Cardiology* 2004: 43(12 Suppl S): 13S-24S.

3. Reaven GM. Banting lecture 1988. Role of insulin resistance in human disease. *Diabetes* 1988: 37(12): 1595-1607.

4. Weiss R, Dziura J, Burgert TS, Tamborlane WV, Taksali SE, Yeckel CW, Allen K, Lopes M, Savoye M, Morrison J, Sherwin RS, Caprio S. Obesity and the metabolic syndrome in children and adolescents. *N Engl J Med* 2004: 350(23): 2362-2374.

5. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet* 2005: 365(9468): 1415-1428.

6. Bogardus C, Lillioja S, Mott DM, Hollenbeck C, Reaven G. Relationship between degree of obesity and in vivo insulin action in man. *The American journal of physiology* 1985: 248(3 Pt 1): E286-291.

7. Ferrannini E, Natali A, Bell P, Cavallo-Perin P, Lalic N, Mingrone G. Insulin resistance and hypersecretion in obesity. European Group for the Study of Insulin Resistance (EGIR). *The Journal of clinical investigation* 1997: 100(5): 1166-1173.

8. McLaughlin T, Allison G, Abbasi F, Lamendola C, Reaven G. Prevalence of insulin resistance and associated cardiovascular disease risk factors among normal weight, overweight, and obese individuals. *Metabolism: clinical and experimental* 2004: 53(4): 495-499.

9. Taraseviciute A, Voelkel NF. Severe pulmonary hypertension in postmenopausal obese women. *European journal of medical research* 2006: 11(5): 198-202.

10. Abenhaim L, Moride Y, Brenot F, Rich S, Benichou J, Kurz X, Higenbottam T, Oakley C, Wouters E, Aubier M, Simonneau G, Begaud B. Appetite-suppressant drugs and the risk of primary pulmonary hypertension. International Primary Pulmonary Hypertension Study Group. *N Engl J Med* 1996: 335(9): 609-616.

11. Delcroix M, Kurz X, Walckiers D, Demedts M, Naeije R. High incidence of primary pulmonary hypertension associated with appetite suppressants in Belgium. *Eur Respir J* 1998: 12(2): 271-276.

12. Rich S, Rubin L, Walker AM, Schneeweiss S, Abenhaim L. Anorexigens and pulmonary hypertension in the United States: results from the surveillance of North American pulmonary hypertension. *Chest* 2000: 117(3): 870-874.

13. Simonneau G, Fartoukh M, Sitbon O, Humbert M, Jagot JL, Herve P. Primary pulmonary hypertension associated with the use of fenfluramine derivatives. *Chest* 1998: 114(3 Suppl): 195S-199S.

14. Dengel DR, Hagberg JM, Pratley RE, Rogus EM, Goldberg AP. Improvements in blood pressure, glucose metabolism, and lipoprotein lipids after aerobic exercise plus weight loss in obese, hypertensive middle-aged men. *Metabolism: clinical and experimental* 1998: 47(9): 1075-1082.

15. Ingelsson E, Sundstrom J, Arnlov J, Zethelius B, Lind L. Insulin resistance and risk of congestive heart failure. *JAMA* 2005: 294(3): 334-341.

16. Li C, Ford ES, McGuire LC, Mokdad AH. Association of metabolic syndrome and insulin resistance with congestive heart failure: findings from the Third National Health and Nutrition Examination Survey. *Journal of epidemiology and community health* 2007: 61(1): 67-73.

17. Witteles RM, Tang WH, Jamali AH, Chu JW, Reaven GM, Fowler MB. Insulin resistance in idiopathic dilated cardiomyopathy: a possible etiologic link. *Journal of the American College of Cardiology* 2004: 44(1): 78-81.

18. Lazar MA. The humoral side of insulin resistance. *Nat Med* 2006: 12(1): 43-44.

19. Combs CK, Johnson DE, Karlo JC, Cannady SB, Landreth GE. Inflammatory mechanisms in Alzheimer's disease: inhibition of beta-amyloid-stimulated proinflammatory responses and neurotoxicity by PPARgamma agonists. *J Neuroscience* 2000: 20(2): 558-567.

20. Humbert M, Monti G, Brenot F, Sitbon O, Portier A, Grangeot-Keros L, Duroux P, Galanaud P, Simonneau G, Emilie D. Increased interleukin-1 and interleukin-6 serum concentrations in severe primary pulmonary hypertension. *Am J Respir Crit Care Med* 1995: 151(5): 1628-1631.

21. Ikeda Y, Yonemitsu Y, Kataoka C, Kitamoto S, Yamaoka T, Nishida K, Takeshita A, Egashira K, Sueishi K. Anti-monocyte chemoattractant protein-1 gene therapy attenuates pulmonary hypertension in rats. *Am J Physiol Heart Circ Physiol* 2002: 283(5): H2021-2028.

22. Martin-Nizard F, Furman C, Delerive P, Kandoussi A, Fruchart JC, Staels B, Duriez P. Peroxisome proliferator-activated receptor activators inhibit oxidized low-density lipoprotein-induced endothelin-1 secretion in endothelial cells. *J Cardiovasc Pharmacol* 2002: 40(6): 822-831.

23. Giaid A, Yanagisawa M, Langleben D, Michel RP, Levy R, Shennib H, Kimura S, Masaki T, Duguid WP, Stewart DJ. Expression of endothelin-1 in the lungs of patients with pulmonary hypertension. *N Engl J Med* 1993: 328(24): 1732-1739.

24. Yudkin JS, Eringa E, Stehouwer CD. "Vasocrine" signalling from perivascular fat: a mechanism linking insulin resistance to vascular disease. *Lancet* 2005: 365(9473): 1817-1820.

25. Stühlinger MC, Abbasi F, Chu JW, Lamendola C, McLaughlin TL, Cooke JP, Reaven GM, Tsao PS. Relationship between insulin resistance and an endogenous nitric oxide synthase inhibitor. *JAMA* 2002: 287(11): 1420-1426.

26. Kielstein JT, Bode-Böger SM, Hesse G, Martens-Lobenhoffer J, Takacs A, Fliser D, Hoeper MM. Asymmetrical dimethylarginine in idiopathic pulmonary arterial hypertension. *Arterioscler Thromb Vasc Biol* 2005: 25(7): 1414-1418.

27. Hansmann G, Wagner RA, Schellong S, Perez VA, Urashima T, Wang L, Sheikh AY, Suen RS, Stewart DJ, Rabinovitch M. Pulmonary arterial hypertension is linked to insulin resistance and reversed by peroxisome proliferator-activated receptor-gamma activation. *Circulation* 2007: 115(10): 1275-1284.

28. Brehm A, Pfeiler G, Pacini G, Vierhapper H, Roden M. Relationship between serum lipoprotein ratios and insulin resistance in obesity. *Clinical chemistry* 2004: 50(12): 2316-2322.

29. McLaughlin T, Abbasi F, Cheal K, Chu J, Lamendola C, Reaven G. Use of metabolic markers to identify overweight individuals who are insulin resistant. *Annals of internal medicine* 2003: 139(10): 802-809.

30. Dokras A, Bochner M, Hollinrake E, Markham S, Vanvoorhis B, Jagasia DH. Screening women with polycystic ovary syndrome for metabolic syndrome. *Obstetrics and gynecology* 2005: 106(1): 131-137.

31. Doehner W, Rauchhaus M, Ponikowski P, Godsland IF, von Haehling S, Okonko DO, Leyva F, Proudler AJ, Coats AJ, Anker SD. Impaired insulin sensitivity as an independent risk factor for mortality in patients with stable chronic heart failure. *Journal of the American College of Cardiology* 2005: 46(6): 1019-1026.

32. Suskin N, McKelvie RS, Burns RJ, Latini R, Pericak D, Probstfield J, Rouleau JL, Sigouin C, Solymoss CB, Tsuyuki R, White M, Yusuf S. Glucose and insulin abnormalities relate to functional capacity in patients with congestive heart failure. *European heart journal* 2000: 21(16): 1368-1375.

33. Swan JW, Anker SD, Walton C, Godsland IF, Clark AL, Leyva F, Stevenson JC, Coats AJ. Insulin resistance in chronic heart failure: relation to severity and etiology of heart failure. *Journal of the American College of Cardiology* 1997: 30(2): 527-532.

34. Dessein PH, Joffe BI. Insulin resistance and impaired beta cell function in rheumatoid arthritis. *Arthritis and rheumatism* 2006: 54(9): 2765-2775.

35. Engelson ES, Agin D, Kenya S, Werber-Zion G, Luty B, Albu JB, Kotler DP. Body composition and metabolic effects of a diet and exercise weight loss regimen on obese, HIV-infected women. *Metabolism: clinical and experimental* 2006: 55(10): 1327-1336.

36. Escarcega RO, Garcia-Carrasco M, Fuentes-Alexandro S, Jara LJ, Rojas-Rodriguez J, Escobar-Linares LE, Cervera R. Insulin resistance, chronic inflammatory state and the link with systemic lupus erythematosus-related coronary disease. *Autoimmunity reviews* 2006: 6(1): 48-53.

37. Lee WC, Chen JJ, Hunt DD, Hou CW, Lai YC, Lin FC, Chen CY, Lin CH, Liao YH, Kuo CH. Effects of hiking at altitude on body composition and insulin sensitivity in recovering drug addicts. *Preventive medicine* 2004: 39(4): 681-688.

38. Paolisso G, Valentini G, Giugliano D, Marrazzo G, Tirri R, Gallo M, Tirri G, Varricchio M, D'Onofrio F. Evidence for peripheral impaired glucose handling in patients with connective tissue diseases. *Metabolism: clinical and experimental* 1991: 40(9): 902-907.

39. Sanchez O, Marcos E, Perros F, Fadel E, Tu L, Humbert M, Dartevelle P, Simonneau G, Adnot S, Eddahibi S. Role of endothelium-derived CC chemokine ligand 2 in idiopathic pulmonary arterial hypertension. *American journal of respiratory and critical care medicine* 2007: 176(10): 1041-1047.

40. Mereles D, Ehlken N, Kreuscher S, Ghofrani S, Hoeper MM, Halank M, Meyer FJ, Karger G, Buss J, Juenger J, Holzapfel N, Opitz C, Winkler J, Herth FF, Wilkens H, Katus HA, Olschewski H, Grunig E. Exercise and respiratory training improve exercise capacity and quality

of life in patients with severe chronic pulmonary hypertension. *Circulation* 2006: 114(14): 1482-1489.

41. Nishiyama Y, Minohara M, Ohe M, Hirai Y, Katoh A, Miyamoto T, Iwami G, Nakata M, Koga Y. Effect of physical training on insulin resistance in patients with chronic heart failure. *Circ J* 2006: 70(7): 864-867.

42. Said SA, Ammar el SM, Suddek GM. Effect of bosentan (ETA/ETB receptor antagonist) on metabolic changes during stress and diabetes. *Pharmacol Res* 2005: 51(2): 107-115.

43. Verma S, Arikawa E, McNeill JH. Long-term endothelin receptor blockade improves cardiovascular function in diabetes. *Am J Hypertens* 2001: 14(7 Pt 1): 679-687.

44. Haffner SM, Greenberg AS, Weston WM, Chen H, Williams K, Freed MI. Effect of rosiglitazone treatment on nontraditional markers of cardiovascular disease in patients with type 2 diabetes mellitus. *Circulation* 2002: 106(6): 679-684.

45. Wang TD, Chen WJ, Lin JW, Chen MF, Lee YT. Effects of rosiglitazone on endothelial function, C-reactive protein, and components of the metabolic syndrome in nondiabetic patients with the metabolic syndrome. *The American journal of cardiology* 2004: 93(3): 362-365.

46. Chu JW, Abbasi F, Lamendola C, McLaughlin T, Reaven GM, Tsao PS. Effect of rosiglitazone treatment on circulating vascular and inflammatory markers in insulin-resistant subjects. *Diab Vasc Dis Res* 2005: 2(1): 37-41.

47. Marx N, Duez H, Fruchart JC, Staels B. Peroxisome proliferator-activated receptors and atherogenesis: regulators of gene expression in vascular cells. *Circulation research* 2004: 94(9): 1168-1178.

48. Hansmann G, de Jesus Perez VA, Alastalo TP, Alvira CM, Guignabert C, Bekker JM, Schellong S, Urashima T, Wang L, Morrell NW, Rabinovitch M. An antiproliferative BMP-2/PPARgamma/apoE axis in human and murine SMCs and its role in pulmonary hypertension. *J Clin Invest* 2008: 118(5): 1846-1857.

Figure 1. Kaplan-Meier 6 Month Event-Free Survival Curve in PAH females. Insulin sensitive (IS, solid line) PAH females have a significantly better outcomes as compared with their insulin resistant (IR, dashed line) counterparts (79% versus 58%, $p<0.05$). Events were defined as death, transplantation, or acute hospitalization due to PAH exacerbation or right heart failure.

TABLE 1: Female Demographic and Metabolic Characteristics

Parameter	**NHANES** (n=967)	**PAH** (n=81)	p*
Age - yr	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 - n (%)			
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 - n (%)	559 (57.8)	35 (43.2)	
Insulin Resistant - n (%)	208 (21.5)	37 (45.7)	<0.0001
Indeterminate - n (%)	200 (20.7)	9 (11.1)	
Cardiovascular Dz - n (%)	58 (6.0)	-	

Data collection in non-diabetic 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.

Table 2: Demographic and Metabolic Profiles of Insulin Sensitive and Insulin Resistant Females

Parameter	NHANES			PAH			p values for NHANES vs PAH		
	Total (n=967)	IS* (n=559)	IR† (n=208)	Total (n= 81)	IS (n=35)	IR (n=37)	Total	IS	IR
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
Triglycerides - mg/dL	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	**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
CV disease - n (%)									
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)	-	-	-	-	-	-

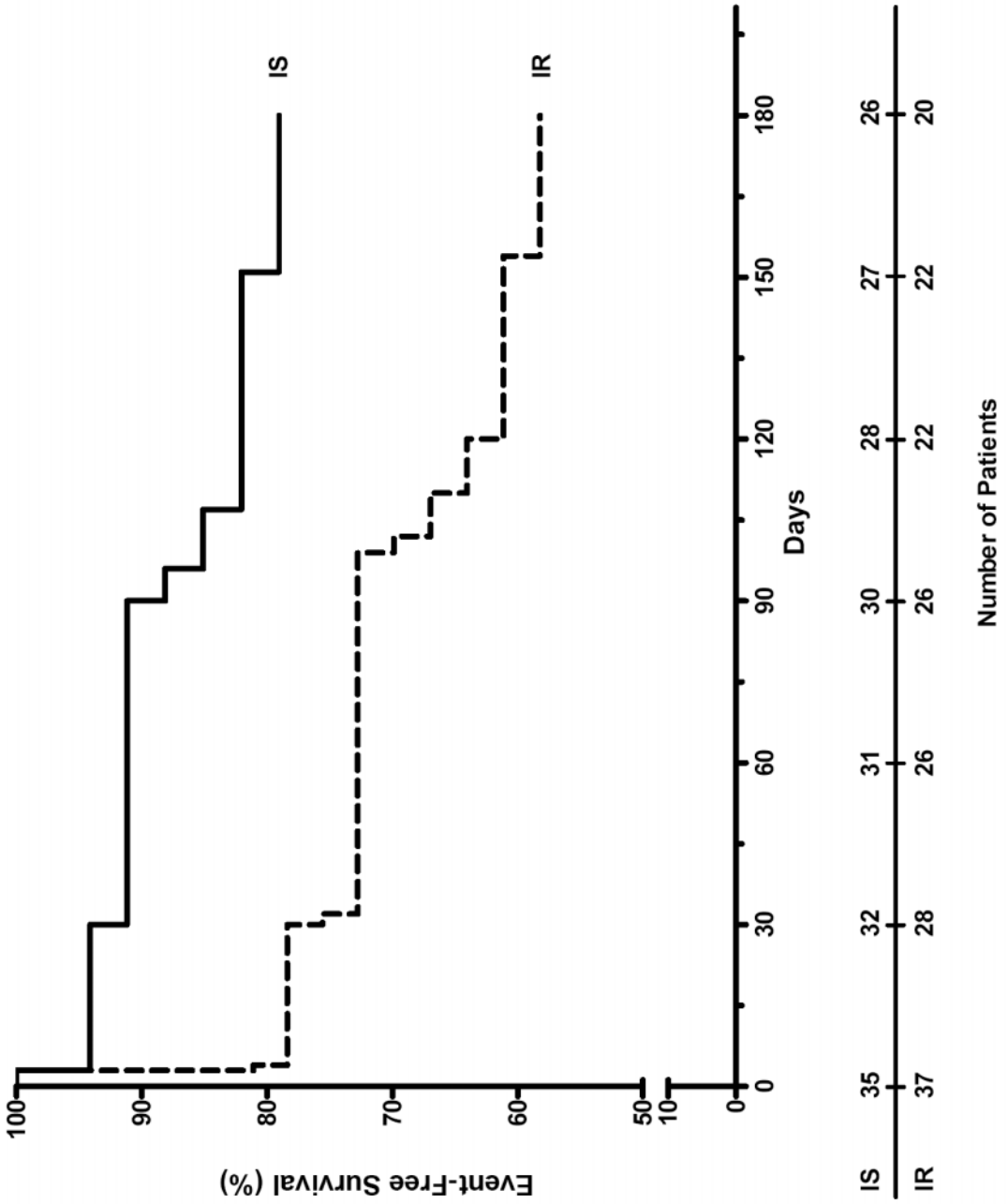
Data collection in non-diabetic female subjects. Values for Age, BMI, & BP indicate mean±SD, all other values are expressed as mean±SEM.
* IS = insulin sensitive and † IR = insulin resistant.
Triglyceride (TG) to high-density lipoprotein cholesterol (HDL-C) ratio (TG/HDL-C) characterizes individuals as insulin sensitive (TG/HDL-C<2) or insulin resistant (TG/HDL-C>3).
p-values are based on the Mann-Whitney U test. For comparison of IR vs. IS in each cohort: ‡ p<0.01, § p<0.001, and || p<0.0001

Table 3: Comparison of Female PAH Patients' Disease Characteristics based on Metabolic Profiles

	Total (n=81)	IS[§] (n=35)	IR[‖] (n=37)	p value
Etiology – n (%) *				
IPAH	28 (34.6)	14 (40.0)	12 (32.4)	>0.05
Stim & Anorex	16 (19.8)	8 (22.8)	5 (13.5)	
CHD	6 (7.4)	0	5 (13.5)	
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 – n (%)				
I	2 (2.5)	1 (2.9)	1 (2.7)	>0.05
II	31 (38.3)	13 (37.1)	15 (40.5)	
III	36 (44.4)	17 (48.5)	13 (35.2)	
IV	10 (12.3)	3 (8.6)	7 (18.9)	
Unknown	2 (2.5)	1 (2.9)	1 (2.7)	
Therapies – n (%) †				
None	28 (34.6)	14 (40.0)	11 (29.7)	>0.05
Monotherapy	37 (45.7)	12 (34.3)	21 (56.8)	
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
Hemodynamics ‡				
mRA - mmHg	9.5±5	9.4±5.1	10.2±5.7	> 0.05
mPAP – 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

All Data are shown as mean±SD. * Etiologies are indicated by idiopathic (IPAH), Stimulant and/or Anorexigen (Stim & Anorex), congenital heart disease (CHD), collagen vascular (CVD), portopulmonary (PPHTN), or human immunodeficiency virus (HIV). † Therapies: Prostanoid = epoprostenol, iloprost, or treprostinil; ETA = Endothelin Antagonist; PDE-I = phosphodiesterase inhibitors. ‡ Hemodynamics: mPAP = mean pulmonary artery pressure, CI =cardiac index, and PVR=pulmonary vascular resistance. § IS = insulin sensitive, || IR = insulin resistant, ** HRT = hormone replacement therapies.

Figure 1


IS
IR
100
90
80
70
60
50
10
0
Event-Free Survival (%)
0
30
60
90
120
150
180
Days
IS 35 32 31 30 28 27 26
IR 37 28 26 26 22 22 20
Number of Patients