ONLINE SUPPLEMENT

Supplemental Methods:

Plasma metabolomics

Fasting plasma was obtained from the cohort described above. Targeted metabolomics was conducted on plasma samples using electrospray ionization liquid chromatography–mass spectrometry (ESI-LC-MS/MS) and MS/MS measurements using the AbsoluteIDQ p180 kit (Biocrates Life Sciences AG, Innsbruck, Austria). This kit allows for the simultaneous quantification of 188 metabolites from different compound classes. These compound classes include 40 acylcarnitines (Cx:y), 21 amino acids (19 proteinogenic amino acids, citrulline and ornithine), 21 biogenic amines, hexose (sum of hexoses–about 90–95% glucose), 90 glycerophospholipids (14 lysophosphatidylcholines (lysoPC) and 76 phosphatidylcholines (PC diacyl (aa) and acyl-alkyl (ae)), and 15 sphingolipids (SMx:y).

The AbsoluteIDQ p180 kit was prepared as previously described[24]. Briefly, 10 μL of plasma sample was pipetted onto the filter inserts of the 96 well kit plate (containing 13C- isotope labeled internal standards). Samples were dried under nitrogen stream and amino acids and biogenic amines were derivatized with 5% phenylisothiocyanate reagent (PITC, 50 μL). Samples were then dried again and metabolites and internal standards were extracted with 5 mM ammonium acetate in methanol. Next, samples were centrifuged through filter membrane two separate dilutions of the final extract were prepared for LC-MS/MS analysis (in MeOH/H2O 1/1) and for FIA-MS/MS analysis (in FIA solvent). MS analyses were

carried out on an API 4000 LC-MS/MS System (ABSciex, Framingham, MA) equipped with 1100 Series HPLC (Agilent Technologies, Palo Alto, CA) using an Agilent Eclipse XDB-C18 (3.5 µm) 3.0 x 100 mm column controlled by Analyst 1.6.2 software. Multiple Reaction Monitoring (MRM) was used for the detection of analytes (amino acids and biogenic amines) and stable labeled internal standards, the latter were used as quantification reference. Lipids, sugars and acylcarnitines were semi-quantified using flow injection analysis (FIA)-MS. The acquired data was processed using Analyst 1.6.2 and MetIDQ (Biocrates Life Sciences AG, Innsbruck, Austria) software. Concentrations of all metabolites were calculated in µM.

Plasma proteomics

Fasting plasma was obtained from the cohort described above. We used the SomaScan aptamer-based platform (SomaLogic, Boulder, CO) that applies a highly multiplexed proteomic technique using single-stranded DNA aptamers to assay protein abundance described previously[25]. The assay was performed as per the manufacturer's protocol[26]. Protein abundance was quantitated and compared using principal components analysis.

Tissue Immunohistochemistry

FFPE slides of human lung were de-paraffinized with two 3-5 minute washes of xylene and re-hydrated with two 3-5 minute washes each of 100, 95, then 70%

ETOH. Slides were then washed 2X for 5 minutes each in CaCl & MgCl free 1X PBS. Heat induced epitope retrieval was performed using a vegetable steamer. Slides were immersed in Tris/EDTA buffer pH 9.0 and steamed at 100°C for 20 minutes, then rinsed with cool tap water for 10 minutes. Slides were washed in 1X TBS w/ 0.025% Triton X 100 for 10 minutes. Blocking was performed with 10% Goat serum/1% BSA in 1X TBS for 2 hours. Slides were drained & a PAP pen was used to encircle tissue sections. Then, primary antibody (Rabbit polyclonal oxLDL orb10973 from Biorbyt, diluted in 1X TBS with 1% BSA) was added at 1:250 dilution to one of two sections per slide, and incubated overnight @ 4°C. The next day, slides were drained & then washed in 1X TBS w/ 0.025% Triton X 100 2X for 5 minutes each. A11037 Alexa Fluor® 594 goat anti-rabbit secondary antibody was added to both sections per slide at 1:800, diluted in 1X TBS with 1% BSA. Slides were incubated in the dark with the secondary @ room temp for 1 hour, rinsed in 1X TBS w/ 0.025% Triton X 100 3X for 5 minutes each, air dried, and then Vectashield (H-1200) Mounting Medium with DAPI was added to sections for visualization of nuclei. Coverslips were applied and slides stored in the dark @ 4°C until imaging. Imaging was done at 20X magnification using a Nikon Eclipse Ti Series confocal microscope.

Supplemental Table 1. Demographic data in PAH and age-, sex- and BMI-matched controls

	Age (years)	Female (n)	BMI (kg/m^2)	Triglyceride (mg/dL)	HDL (mg/dL)	LDL (mg/dL)
PAH N=10	44.7±6.4	5	30.6±1.1	136.0±14.1	36.1±0.1	107.8±0.8
Control N=30	43.7±7.5	15	31.3±2.1	130.4±3.9	40.3±5.9	119.0±11.2

Supplemental Table 2. Metrics of Insulin Resistance and Glucose Intolerance

Patient Number	BMI (Kg/M^2)	Pre-diabetes	Impaired glucose tolerance	Insulin resistant (HOMA-IR)	Insulin resistant (HDL:TG ratio)
1	28.5			X	X
2	31.9				X
3	27.4	X			X
4	28.1	X	X		X
5	31.7		X		X
6	30.4	X	X	X	X
7	23.6			X	X
8	32.1		X		X
9	30.6	X	X	X	X
10	40.8				X

X = present

Supplemental Table 3. Demographic info for patients included in proteomics, metabolomics and lipoprotein analysis

Demographics and Clinical Characteristics of PAH Patients and Controls in the Study		
	PAH Patients (n = 28)	Controls (n = 37)
Age	49.0 ± 12.4	45.0 ± 9.9
Sex (Female)	23 (82%)	31 (82%)
BMI	29.5 ± 7.8	28.4 ± 7.1
Hypertension (%)	8 (29%)	7 (19%)
Diabetes (%)	4 (14%)	2 (5%)
Hyperlipidemia (%)	2 (7%)	4 (11%)
PAH Type		
HPAH	6 (21%)	
IPAH	20 (71%)	
APAH	2 (7%)	
Baseline Values		
Functional Class	3.1 ± 0.6	
6MWD (m)	341 ± 105	
Mean PA Pressure (mmHg)	58.2 ± 11.0	
PCWP(mmHg)	9.6 ± 4.2	
PVR (Wood units)	14.7 ± 6.9	
Cardiac Output (L/min)*	3.74 ± 1.25	
Values nearest to blood draw date		
Functional Class	2.3 ± 0.8	
6MWD (m)	388 ± 132	
Mean PA Pressure (mmHg)	53.4 ± 13.0	
PCWP (mmHg)	12.4 ± 8.1	
PVR (Wood units)	10.6 ± 5.3	
Cardiac Output (L/min)*	4.2 ± 1.3	

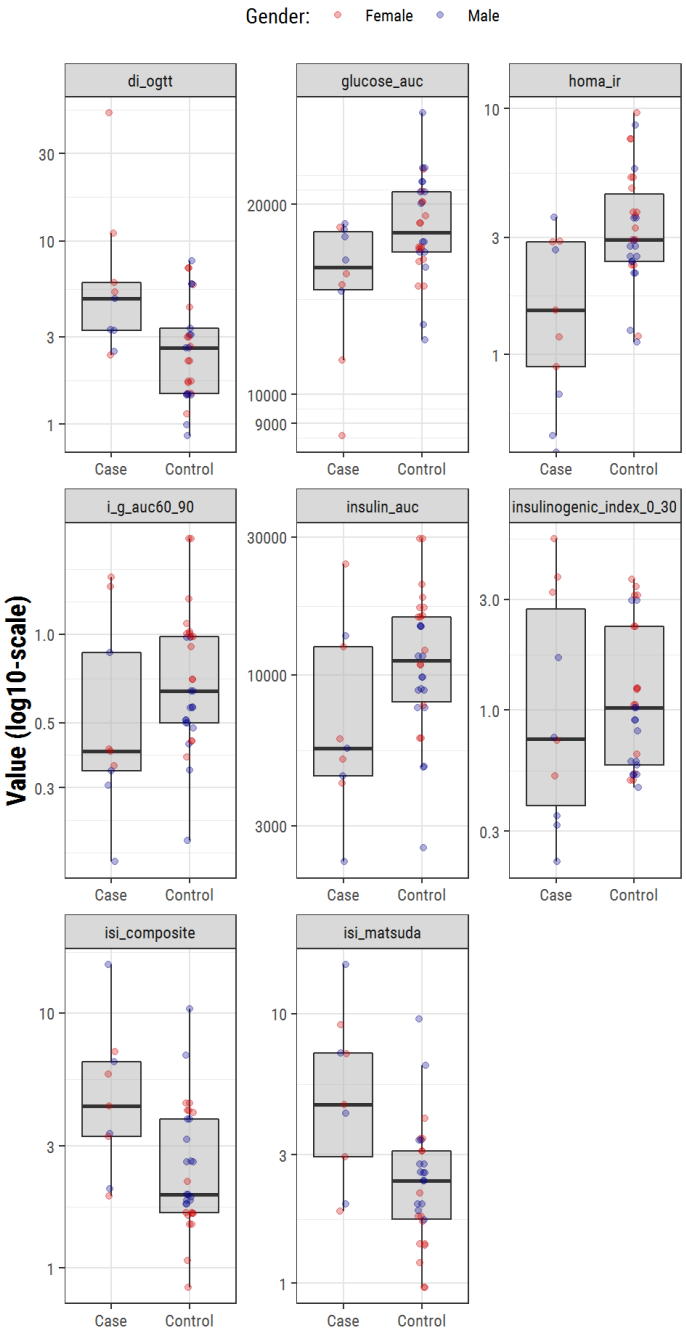
*The Fick method for measuring cardiac output was used preferentially, and thermodilution substituted if Fick measurements not available.
HPAH = heritable PAH, IPAH = idiopathic PAH, APAH = associated PAH, 6MWD = six-minute walk distance, PCWP = pulmonary capillary wedge pressure, PVR = pulmonary vascular resistance.

Supplemental Table 4. Clinical Characteristics of Insulin Resistant vs. non-Insulin Resistant by Lipoprotein-based Insulin Resistance Score

	LPIRS ≥ 48 (n=9)	LPIRS <48 (n=12)	p value
Age (years)	42.3 ± 7.8	53.2 ± 13.6	0.04
Sex (m/f)	1/8	2/10	NS
BMI (kg/M^2)	29.1 ± 7.2	30.3 ± 9.1	NS
PAH type Idiopathic Heritable	 8 1	 11 1	NS
Functional Class	3.1 ± 0.4	3.1 ± 0.8	NS
mPAP (mmHg)	59.6 ± 3.5	58.3 ± 13.2	NS
PVR (Wood Units)	17.7 ± 6.8	13.7 ± 7.2	NS
6 minute walk distance (m)	400 ± 116.7	422 ± 111.3	NS
Lipoprotein Inflammation Marker	443.8 ± 62.3	401.3 ± 84.5	0.08

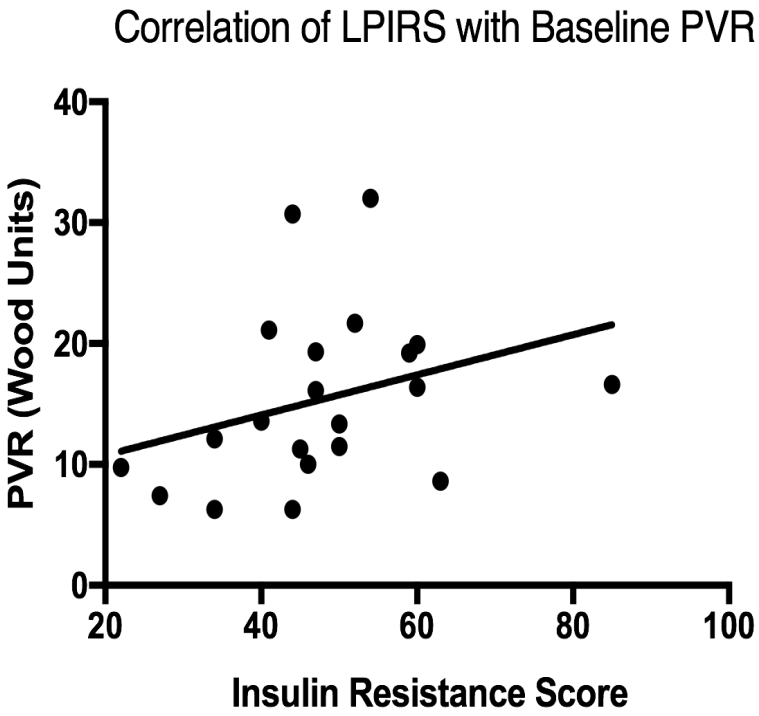
Data presented as mean ± SD
mPAP = mean pulmonary arterial pressure
PAH = pulmonary arterial hypertension
PVR = pulmonary vascular resistance
CO = cardiac output
PWP = pulmonary wedge pressure

Supplemental Figure 1.



Supplemental Figure 1. Oral Glucose Tolerance Testing (OGTT) Metrics in PAH (n=10) and age-, sex- and BMI-matched controls (n=30). AUC=area under the curve, ISI=insulin sensitivity index, HOMA-IR=homeostatic model assessment-insulin resistance, DI=disposition index,p<0.05 for di-OGTT, glucose AUC, HOMA-IR, insulin AUC, isi-composite and isi-Matsuda.

Supplemental Figure 2



Supplemental Figure 2. There was a weak correlation (p=0.05) by Spearman's r (0.42) between pulmonary vascular resistance (PVR) at diagnosis and lipoprotein-based insulin resistance score. n=21 PAH patients.