Thoracic Visceral Adipose Tissue Area and Pulmonary Hypertension in Lung Transplant Candidates: The Lung Transplant Body Composition Study

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Abstract:

Rationale: Obesity is associated with an increased risk of pulmonary hypertension (PH), however regional adipose tissue deposition is heterogeneous with distinct cardiovascular phenotypes.

Objective: To determine the association of body mass index (BMI), thoracic visceral and subcutaneous adipose tissue areas (VAT and SAT, respectively) with PH in patients with advanced lung disease referred for lung transplantation.

Methods: We studied patients undergoing evaluation for lung transplantation at 3 centers from the Lung Transplant Body Composition Study. PH was defined as mean pulmonary artery pressure > 20 mmHg and pulmonary vascular resistance (PVR) ≥ 3 Wood units. VAT and SAT were measured on chest computed tomography and normalized to height squared.

Results: 137 (34%) of 399 patients included in our study had PH. Doubling of thoracic VAT was associated with significantly lower PVR (β -0.24, 95%CI -0.46, -0.02, p = 0.04), higher PAWP (β 0.79, 95% CI 0.32, 1.26, p = 0.001), and decreased risk of PH (RR 0.86 95%CI 0.74-0.99, p = 0.04) after multivariate adjustment. Vaspin levels were higher in patients without PH (median 101.8 vs 92.0 pg/mL, p <0.001), but did not mediate the association between VAT and the risk of PH. SAT and BMI were not independently associated with risk of PH.

Conclusions: Lower thoracic VAT was associated with a higher risk of PH in patients with advanced lung disease undergoing evaluation for lung transplantation. The role of adipokines in the pulmonary vascular disease remains to be evaluated.

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Obesity has been implicated as a risk factor for several types of pulmonary hypertension (PH)(1), and almost two-thirds of patients with PH are overweight or obese at the time of diagnosis(2, 3). PH occurs in approximately 20% of patients with parenchymal lung disease(4-6) and increases the risk of death in these patients(7-9). The link between obesity and the development of PH is not well understood. Obesity is a heterogeneous disease with multiple fat deposition phenotypes, which have distinct cardiovascular effects(10-13). For example, abdominal visceral adipose tissue area (VAT) is particularly metabolically active and is associated with greater risk of cardiovascular disease compared to subcutaneous adipose tissue area (SAT)(14, 15). VAT secretes various adipokines leading to endothelial dysfunction(16-18).

In the thorax, epicardial and pericardial fat comprise the main source of VAT, but the two fat depots are anatomically and embryologically distinct with unique characteristics(19). Epicardial fat was traditionally considered to be white adipose tissue (similar to abdominal VAT), although more recent evidence suggests that epicardial fat more closely resembles brown adipose tissue with the highest rates of lipid-consuming and fatty acid metabolism(19-21). Epicardial fat (unlike other fat depots) is contiguous with the myocardium with no fascia separation with strong evidence for paracrine effects on the coronary microcirculation(19, 22, 23). Pericardial fat, on the other hand, is located anteriorly to the epicardial fat between the visceral and parietal pericardium and is a source of proinflammatory cytokines(24, 25). Pericardial fat has been associated with adverse cardiovascular outcomes and coronary artery calcifications(26, 27). Thoracic adipose tissue is of particular interest in pulmonary disease, especially PH, because its lymphatics drain directly into the pulmonary circulation and it can exert local and systemic effects(28).

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While most adipokines promote inflammatory and atherogenic processes, there are some adipokines which have a more favorable cardiovascular profile, such as vaspin(29). Vaspin inhibits endothelial cell apoptosis caused by increased free fatty acids and promotes endothelial nitric oxide synthase (eNOS) with resulting vasodilation(30, 31).

Collectively, these data highlight the complex association of obesity with PH. While obesity may be associated with PH, intrathoracic VAT may actually be protective via production of adipokines such as vaspin which has vasodilatory properties and could impact the nitric oxide pathway. We sought to determine the association of body mass index (BMI), intrathoracic VAT and SAT with PH in patients with advanced lung disease being evaluated for lung transplantation. We hypothesized that higher BMI and lower thoracic VAT would be associated with a higher risk of PH.

Methods

Study Population

We performed a cross-sectional analysis of the Lung Transplant Body Composition (LTBC) study(32), a prospective multicenter cohort study investigating the mechanistic links between adiposity and primary graft dysfunction after lung transplantation. The LTBC study enrolled adult patients age greater than or equal to 18 years with advanced lung disease evaluated for lung transplantation at Duke University, Columbia University Medical Center, and the University of Pennsylvania between 2011 and 2013(33). For this study we included patients from LTBC who had a diagnosis of interstitial lung disease (ILD), chronic obstructive pulmonary diseases

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(COPD), sarcoidosis or pulmonary arterial hypertension (PAH) and who underwent chest computed tomography (CT) examination as part of the LTBC protocol. We excluded patients with cystic fibrosis (CF), non-CF bronchiectasis, or a prior lung transplantation as PH in the setting of chronic inflammation from infection or long standing calcineurin inhibitors and corticosteroid use may have a different etiology from other forms.

PH was defined according to the 6th World Symposium on Pulmonary Hypertension (WSPH) recommendations as mean pulmonary artery pressure (mPAP) > 20 mm Hg and a pulmonary vascular resistance (PVR) ≥ 3 Wood units as determined by right heart catheterization (RHC) at the time of lung transplant evaluation(34). We performed a secondary analysis after excluding patients with elevated pulmonary arterial wedge pressure (PAWP) (> 15 mm Hg). Participants provided written informed consent for participation in the LTBC study. This study was approved by the institutional review board of the University of Pennsylvania.

CT Measurements of Fat Depots

LTBC study participants underwent chest imaging during full inspiration performed with multi-detector row CT scanners at the time of evaluation for lung transplantation. Details of chest fat quantification have been previously described(35-37). The thoracic SAT-VAT interface was defined as the interior surface of the rib cage; fat within this surface was considered VAT and that external to this surface was considered SAT for all slices which are superior to the diaphragm. Using a standardized anatomical space approach which was originally developed and validated in a cohort of lung transplant candidates, the thoracic VAT was measured at the mid-T7 vertebral level as this has been previously shown to have maximum correlation with

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total volume of thoracic VAT (r = 0.86) (Figure 1A)(35). The thoracic SAT was measured at the mid-T8 vertebral level which has shown maximum correlation with total volume of thoracic SAT (r = 0.97) (Figure 1B)(35). The primary exposure variables were BMI and thoracic VAT and SAT. We indexed each of the VAT and SAT areas to height squared (m^2)(37, 38).

Covariates

Demographic variables and clinical parameters were collected prospectively including age, sex, race/ethnicity, spirometric volumes, and six-minute walk distance (6MWD). Weight was measured wearing light indoor clothing and no shoes to the nearest 0.1 kg. Height was measured with a wall-mounted stadiometer to the nearest 0.1 cm.

Vaspin Measurement

Phlebotomy was performed at the lung transplant evaluation visit and blood was collected into citrate tubes. We measured plasma vaspin in duplicate in citrated samples stored at -80 °C using a commercially available ELISA (R&D Systems, Minneapolis, MN). The intra-assay coefficient of variation was 2.17%. As our analysis was ancillary to the LTBC study, we only had available samples on a subset of patients (n = 78) which was independent of their PH status.

Statistical Analysis

Baseline characteristics of study participants were compared using two-tailed Student's T-test, Wilcoxon rank sum test, and Fisher's exact test, as appropriate. Spearman correlation was used to test the association between adipose measures and weight and BMI. These associations

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were then tested for non-linear effects by using quadratic terms for weight and BMI using polynomial regression. Logistic regression models were used to assess the crude association between VAT and SAT and the odds of PH. Models were adjusted for *a priori* confounders including age, sex, race/ethnicity, primary lung diagnosis, forced vital capacity (FVC), and PAWP. We then transformed odds ratios to relative risks (RR) using the *orsk* package(39). We performed a sensitivity analysis of generating RRs using a modified Poisson regression(40).

Generalized additive models with loess smoothing functions were used to demonstrate the non-linear association between adipose tissue measures and risk of PH. Similarly, linear regression models were used to assess the association between hemodynamic measures (dependent variables) and the adipose measures (independent variable) in the entire cohort (with and without PH) and adjusted these models for the same confounders. We generated 5 datasets using multiple imputation by chained equations to handle missing data using the *mice* package in R(41).

We performed sensitivity analysis after restricting the study sample to those with normal PAWP, after restricting the sample to those without PAH, and checked for effect modification by underlying lung disease diagnosis. We also performed mediation analyses to explore whether vaspin mediated the association of adipose tissue with PH using nonparametric bootstrapping estimation methods using the *mediation* package in R(42). All analyses were performed using R 2018 version 3.6.1 (Vienna, Austria).

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Results

Patient Demographics

There were 530 patients in the LTBC study, 514 of which had interpretable chest CT scans (Figure 2). After excluding patients with a diagnosis of CF, non-CF bronchiectasis and those who had previously received lung transplantation as well as those who either did not have a right heart catheterization or who were missing hemodynamic measurements, 399 patients were included in our final study sample. Of these, 137 subjects (34%, 95% CI 29%-39%) had PH (Table 1). There were no differences in age, or sex between patients with and without PH. There was also no difference in BMI between patients with and without PH (25.7 ± 4.5 vs 25.8 ± 4.1 kg/m^2, p =0.82). Patients with PH were more likely to be non-Hispanic black or Asian than those patients without PH. Patients with PH were more likely to have higher forced expiratory volume in one second (FEV1) and more commonly identified as needing some assistance rather than full assistance. Not surprisingly, patients with PH had lower 6MWD, higher PVR and lower cardiac index.

Association of Thoracic Visceral and Subcutaneous Adipose Tissues with Body Mass Index

Thoracic VAT was non-linearly associated with BMI (Figure 3A, p for quadratic trend 0.01). Similarly, thoracic SAT was non-linearly associated with BMI (Figure 3B, p for quadratic trend 0.04).

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Association between Thoracic Visceral and Subcutaneous Adipose Tissue and Hemodynamic Variables

Doubling of the thoracic VAT area was associated with significantly lower PVR (β -0.24, 95%CI -0.46, -0.02, p = 0.04) and higher PAWP (β 0.79, 95% CI 0.32, 1.26, p = 0.001) after adjustment for age, sex, race/ethnicity, underlying lung disease and FVC in the entire study cohort (Table 2). A doubling of thoracic SAT area was also independently associated with lower PVR and higher PAWP, although thoracic SAT was not associated with prevalence of PH.

A one kg/m^2 increase in BMI was independently associated with higher mPAP, lower PVR, higher PAWP and lower cardiac index. However, there was no association of BMI with the probability of PH (RR = 1.00, 95%CI 0.97-1.04, p = 0.98).

Association between Thoracic Visceral and Subcutaneous Adipose Tissue and Risk of Pulmonary Hypertension

Higher thoracic VAT area was associated with decreased risk of PH (RR per doubling of VAT area = 0.88 95% CI 0.78-0.99, p = 0.03) (Table 3) which persisted after adjustment for age, sex, race/ethnicity, FVC, lung disease diagnosis, and PAWP (Table 3 and Figure 4). The association of thoracic VAT and PH held even after additional adjustment for BMI. Thoracic SAT and BMI were not associated with risk of PH in this cohort (Table 3). The associations observed in the full cohort were maintained in a sensitivity analysis restricted to patients with normal PAWP and restricted to those without PAH after adjustment for age, sex, race/ethnicity, FVC, and lung disease diagnosis (RR per doubling of thoracic VAT area = 0.86 95% CI 0.74-0.99, p = 0.05 and RR per doubling of thoracic VAT area = 0.84 95% CI 0.72-0.98, p = 0.03, respectively). The use of

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binomial modified Poisson regression resulted in RRs which were identical to those in our main analysis.

There was no significant effect modification by underlying lung disease of the association between thoracic VAT and PH risk (p for interaction = 0.16). After limiting the study population to subjects with ILD (n = 239) or COPD (n = 113), we observed comparable results (but with wider 95%CI due to smaller sample size, as expected) (data not shown).

Association between Thoracic Visceral Adipose Tissues, Vaspin and Risk of Pulmonary Hypertension

Blood samples were available for 78 subjects. Circulating vaspin levels were positively correlated with thoracic VAT (r 0.38, p = 0.001) but not with thoracic SAT (r 0.02, p = 0.86) nor with BMI (r 0.08, p 0.47). Vaspin levels were higher in the patients without PH as compared to those with PH (median [IQR] 101.8 [66.5-266.5] vs 92.0 [57.2-175.8] pg/mL, p <0.001, Figure 5). However, circulating vaspin levels did not appear to mediate the association between thoracic VAT and the risk of PH (indirect effect 1.0%).

Discussion

We found that higher thoracic VAT area was independently associated with decreased risk of PH in patients with COPD, ILD, sarcoidosis and PAH undergoing evaluation for lung transplantation. This association persisted in the subset of patients with normal PAWP and was not modified by the underlying lung diagnosis. Higher thoracic VAT was also independently

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associated with lower PVR and higher PAWP. Even though thoracic SAT was also associated with PVR and PAWP, it was not independently associated with the risk of PH. Thoracic VAT and SAT were non-linearly associated with BMI, which was not associated with risk of PH.

While VAT is most commonly associated with increased cardiovascular risk and endothelial dysfunction, higher thoracic VAT was associated with a decreased risk of PH in our study. Thoracic VAT, particularly epicardial adipose tissue, has unique properties that distinguishes it from other VAT depots because of a delicate equilibrium between protective and harmful cardiovascular effects. Epicardial fat has been postulated to function similarly to brown adipose tissue, with cardioprotection in the setting of hypothermia and hypoxia(19). Epicardial adipose tissue expresses genes associated with brown adipose tissue including uncoupling protein-1 (*UCP1*) which is absent in other fat depots such as SAT(20). Epicardial fat secretes cardioprotective adipokines, such as adiponectin, adrenomedullin and vaspin(19, 43-46) which could be contributing to the decrease in PVR associated with higher thoracic VAT. Vaspin is a serine protease inhibitor that has protective effects on the vascular endothelium by inhibiting free-fatty acid induced apoptosis of endothelial cells and increasing the effect of nitric oxide (NO) and endothelial nitric oxide synthase mediated by human signal transducer and activator of transcription 3(30, 47). The NO signaling pathway is thought to play a major role in the pathogenesis of pulmonary hypertension(48-51).

The association between increased levels of thoracic VAT and decreased risk of PH in our cohort could be potentially explained by decreased pulmonary vasoconstriction or vascular remodeling. In our cohort, we found that circulating vaspin levels were positively correlated with thoracic VAT and were lower in patients with PH. The serum vaspin levels measured in our

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cohort were on the lower spectrum of reported serum vaspin ranges(52). Serum vaspin levels are impacted by obesity and impaired glucose tolerance. We were not able to show that the association between thoracic VAT and PH is mediated by circulating vaspin levels. A single sample of the peripheral circulation may not reflect local vaspin levels over time and we only had samples on a small subset of our study sample, so this adipokine (or others) could still play a role in this relationship.

Higher BMI has been shown to be positively correlated with increased PAWP likely as a result of increased intrathoracic pressure in the obese(53). In our study, BMI as well as VAT and SAT were associated with higher PAWP. However, this study demonstrates that BMI itself does not sufficiently account for the heterogeneity of fat depots and deposition(54). In fact, thoracic VAT and SAT were not linearly associated with BMI. Even though we found an association between higher BMI and higher mPAP, higher PAWP, and lower PVR, BMI was not independently associated with the risk of pulmonary hypertension whereas thoracic VAT was, even after adjustment for BMI. One possible explanation is in the requirement of PVR $\geq$ 3 Wood units in the 6th WSPH definition of PH which many patients in our cohort did not meet as they had mild elevations in mPAP but low or normal PVR. Another possible explanation is a truncated BMI distribution excluding the morbidly obese as patients were referred for a lung transplant evaluation. Our work supports the need for improved phenotyping of obesity and fat distribution in patients with PH to allow for improved personalization of treatments, a priority in pulmonary vascular disease research(55).

Our study has limitations. Our study measured intrathoracic VAT but did not differentiate between epicardial fat and pericardial fat. While our hypothesis is based on the role of epicardial fat which is known to have paracrine and endocrine effects on the heart, our results refer to overall intrathoracic VAT. The cross-sectional design which does not permit inferences regarding the temporal sequence/causality between adipose measures and development of PH. We included a heterogeneous population of patients with advanced lung disease presenting for lung transplant evaluation at multiple centers which increases our generalizability; however, our findings may not apply to patients with less severe lung disease or who are not suitable candidates for lung transplant evaluation. Obesity is a relative contraindication to lung transplantation, potentially leading to referral bias. Additionally, many of the subjects included in our cohort could have been on corticosteroid therapy which could impact glucose tolerance and vaspin levels. Thus it is unclear if our findings hold true for patients with higher BMI. Finally, we adjusted our models for several variables that could affect the relationship between adipose tissue and PH, although the possibility of residual or unmeasured confounding effects remains.

In conclusion, we found that lower thoracic VAT area was associated with a higher risk of PH in patients with advanced lung disease undergoing evaluation for lung transplantation, an association that was not observed with thoracic SAT. Our results support the need for deep phenotyping of obesity to better understand its association with PH and explore novel therapeutic avenues.

ANNALSATS Articles in Press. Published June 12, 2020 as 10.1513/AnnalsATS.202003-247OC

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Figure Legends:

Figure 1: Thoracic adipose tissue delineation at the level of mid-thorax. A: Thoracic visceral adipose tissue (green area) B. Thoracic subcutaneous adipose tissue (green area)

Figure 2: Flowchart of study inclusion

Figure 3: A. Non-linear association between thoracic visceral adipose tissue and body mass index. The 95% confidence intervals were depicted in gray shade. B. Non-linear association between thoracic subcutaneous adipose tissue and body mass index. The 95% confidence intervals were depicted in gray shade.

Figure 4: Continuous associations between visceral adipose tissue area and the prevalence of pulmonary hypertension using a generalized additive model Thick dotted lines: smoothed regression lines adjusted to age, sex, race/ethnicity, lung disease diagnosis, forced vital capacity, pulmonary artery wedge pressure. Thin solid lines: 95% confidence intervals.

Figure 5: Median vaspin levels and PH status ($p < 0.001$). Median and interquartile range are shown. PH (n = 24) and Non-PH (n = 54).

ANNALSATS Articles in Press. Published June 12, 2020 as 10.1513/AnnalsATS.202003-247OC

Table 1: Baseline characteristics of patients by pulmonary hypertension status

	No PH (n=262)	PH (n=137)
Age, years	61 ± 9	59 ± 11
Female sex, %	38	46
Race/Ethnicity, %		
Non-Hispanic white	84	66
Non-Hispanic black	9	20
Hispanic	4	5
Asian	2	7
Other/multiracial	1	2
Body mass index, kg/m^2	25.8 ± 4.1	25.7 ± 4.5
Primary lung diagnosis, %		
Interstitial lung disease	59	63
Chronic Obstructive Pulmonary Disease	35	15
Pulmonary arterial hypertension	--	12
Sarcoidosis	4	7
Other	2	3
Forced expiratory volume in 1 second, % pred (n= 371)	42 ± 21	47 ± 21
Forced vital capacity, % pred (n= 371)	53 ± 18	53 ± 21
6-minute walk distance, m (n= 397)	351 (252-427)	287 (201-382)
Functional status, % (n= 246)		
Needs no assistance	--	1
Needs some assistance	32	48
Needs full assistance	68	51
Hemodynamics		
Mean pulmonary artery pressure, mm Hg	20 (17-24)	33 (27-40)
Pulmonary artery wedge pressure, mm Hg	10 (7-14)	9 (6-13)
Pulmonary vascular resistance, Wood units	1.9 (1.3-2.4)	4.7 (3.8-6.9)
Cardiac output, L/min	5.29 (4.60-6.16)	4.51 (3.92-5.30)
Cardiac index, L/min/m^2	2.80 (2.44-3.19)	2.49 (2.14-2.85)

Mean ± SD or median (interquartile range)

ANNALSATS Articles in Press. Published June 12, 2020 as 10.1513/AnnalsATS.202003-247OC

Table 2: Association between thoracic visceral and subcutaneous adipose tissue measures and hemodynamics

	mPAP, mm Hg			PVR, Wood units			PAWP, mm Hg			CI, L/min/m^2		
	β	**95% CI**	**p**	**β**	**95% CI**	**p**	**β**	**95% CI**	**p**	**β**	**95% CI**	**p**
Doubling of VAT cm^2/m^2	0.08	-0.79, 0.95	0.86	-0.24	-0.46, -0.02	0.04	0.79	0.32, 1.26	0.001	-0.02	-0.08, 0.04	0.48
Doubling of SAT cm^2/m^2	0.70	-0.03, 1.71	0.17	-0.32	-0.57, -0.06	0.02	1.11	0.56-1.65	<0.001	-0.03	-0.10, 0.04	0.38
Body mass index per 1 kg/m^2 increase	0.33	0.11, 0.56	0.003	-0.04	-0.09, 0.02	0.02	0.33	0.21, 0.45	<0.001	-0.03	-0.04, -0.01	<0.001

Adjusted for age, sex, race/ethnicity, lung disease diagnosis, forced vital capacity

β: standardized regression coefficient; 95% CI: 95% confidence interval; mPAP: Mean pulmonary artery pressure; PVR: Pulmonary vascular resistance; PAWP: Pulmonary artery wedge pressure; CI: Cardiac index; VAT: Visceral adipose tissue; SAT: Subcutaneous adipose tissue

ANNALSATS Articles in Press. Published June 12, 2020 as 10.1513/AnnalsATS.202003-247OC

Table 3: Unadjusted and adjusted Relative Risks for the presence of pulmonary hypertension

	Model 1		Model 2		Model 3		Model 4	
	RR (95% CI)	**p**	**RR (95% CI)**	**p**	**RR (95% CI)**	**p**	**RR (95% CI)**	**p**
Doubling of VAT cm^2/m^2	0.88 (0.78-0.99)	0.03	0.84 (0.72-0.97)	0.02	0.86 (0.74-0.99)	0.04	0.83 (0.70-0.97)	0.02
Doubling of SAT cm^2/m^2	0.96 (0.84-1.09)	0.56	0.90 (0.76-1.06)	0.21	0.92 (0.78-1.09)	0.36	0.84 (0.63-1.08)	0.17
1 kg/m^2 increase in BMI	0.99 (0.97-1.03)	0.81	0.99 (0.96-1.03)	0.63	1.00 (0.97-1.04)	0.98	--	--

Model 1: Unadjusted

Model 2: Adjusted for age, sex, race/ethnicity, lung disease diagnosis, forced vital capacity

Model 3: Adjusted for age, sex, race/ethnicity, lung disease diagnosis, forced vital capacity, pulmonary artery wedge pressure

Model 4: Adjusted for age, sex, race/ethnicity, lung disease diagnosis, forced vital capacity, pulmonary artery wedge pressure, body mass index

VAT: Visceral adipose tissue area; SAT: Subcutaneous adipose tissue area; BMI: body mass index; RR: Relative Risk; 95% CI: 95% Confidence interval

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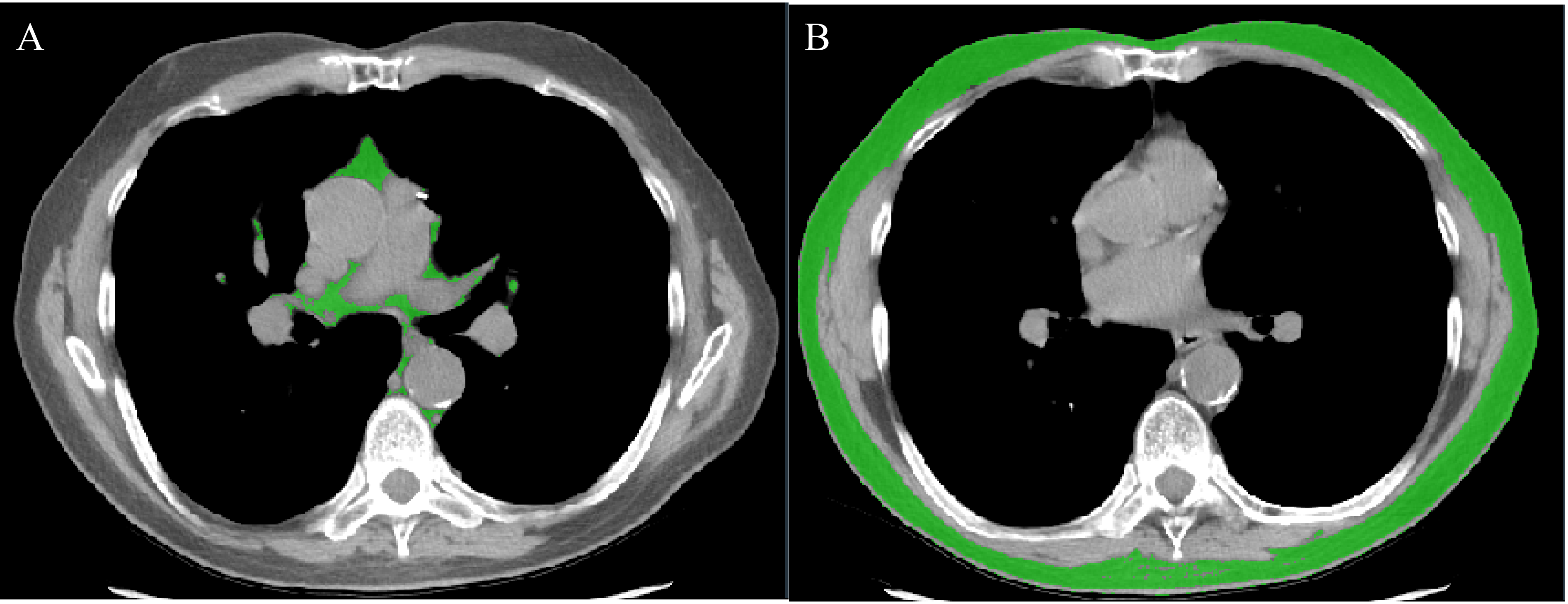
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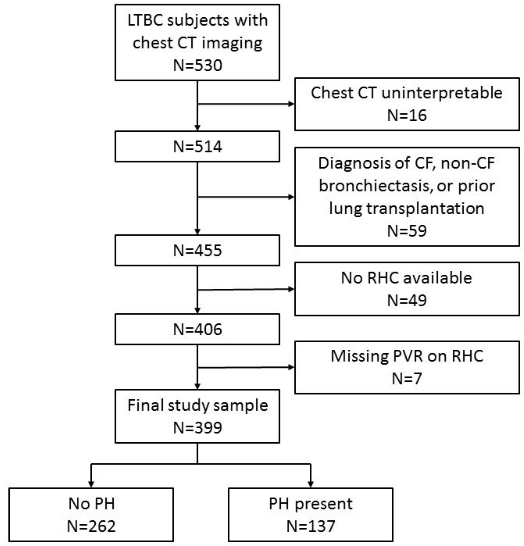

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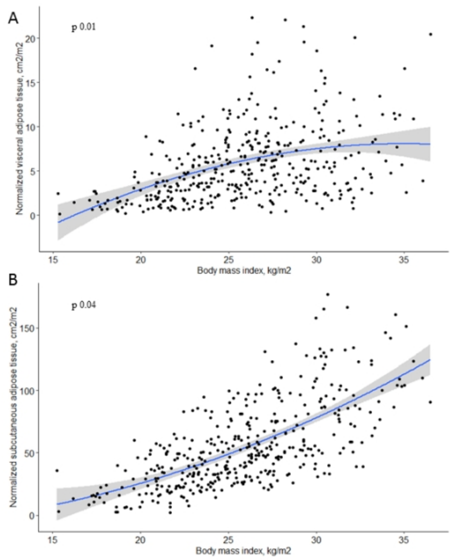

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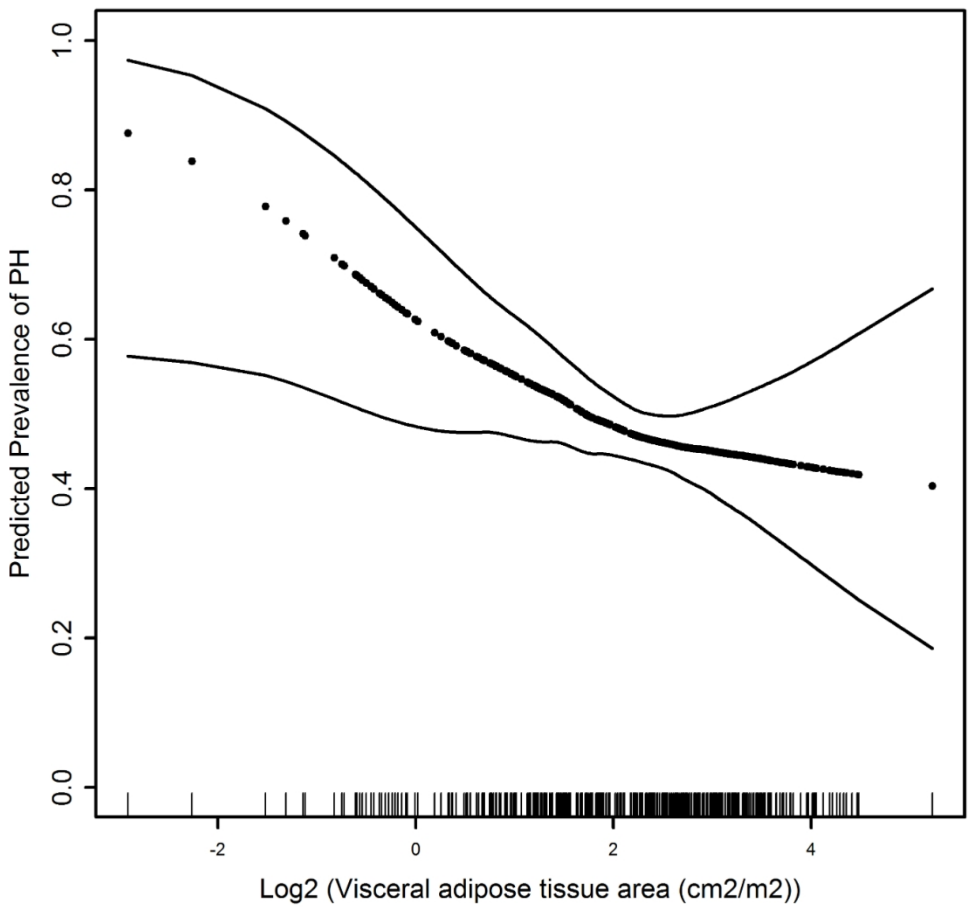

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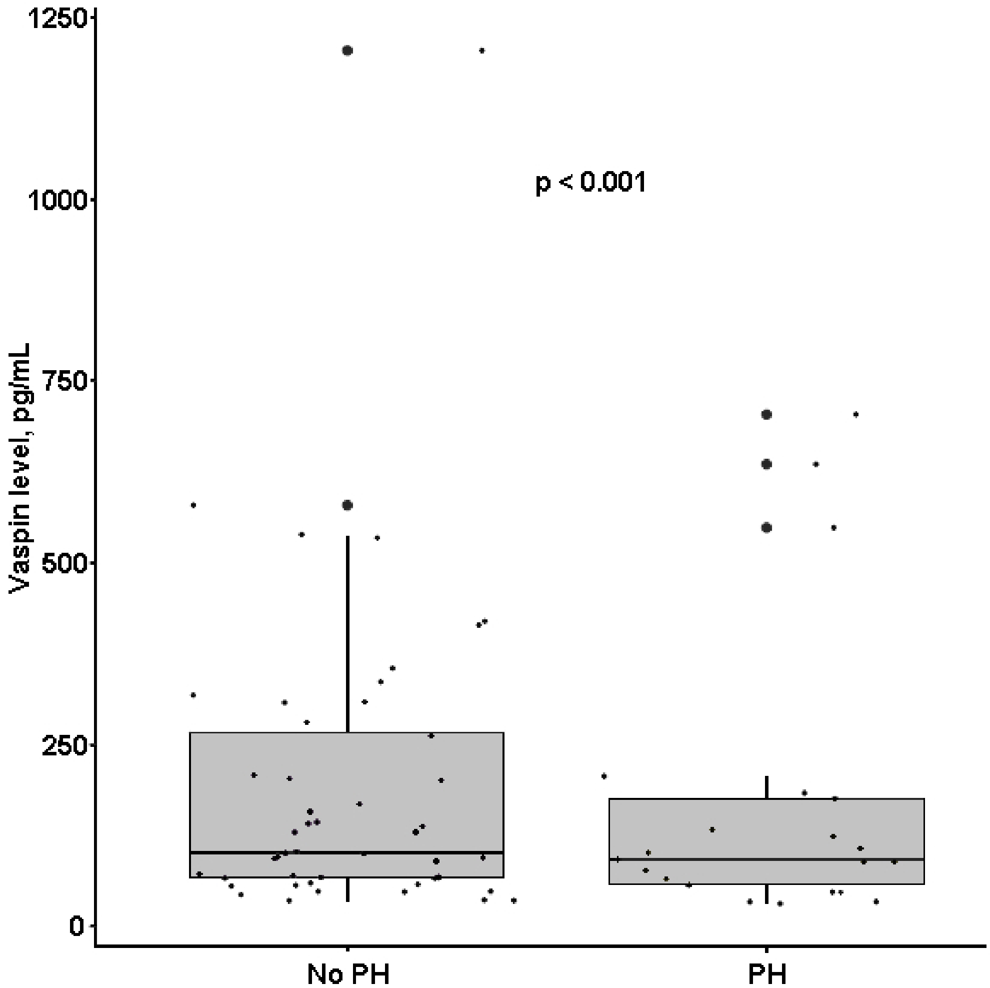

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