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Authors

Kim, John S

Anderson, Michaela R

Podolanczuk, Anna J

et al.

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Associations of Serum Adipokines With Subclinical Interstitial Lung Disease Among Community-Dwelling Adults

The Multi-Ethnic Study of Atherosclerosis (MESA)

John S. Kim, MD; Michaela R. Anderson, MD; Anna J. Podolanczuk, MD; Steven M. Kawut, MD; Matthew A. Allison, MD, MPH; Ganesh Raghu, MD; Karen Hinckley-Stuckovsky, MS; Eric A. Hoffman, PhD; Russell P. Tracy, PhD; R. Graham Barr, MD, DrPH; David J. Lederer, MD; and Jon T. Giles, MD, MPH

BACKGROUND: Adipokines have inflammatory and fibrotic properties that may be critical in interstitial lung disease (ILD). We examined associations of serum adipokine levels with CT imaging-based measures of subclinical ILD and lung function among community-dwelling adults.

METHODS: A subset of the original Multi-Ethnic Study of Atherosclerosis cohort (n = 1,968) had adiponectin, leptin, and resistin measured during follow-up visits (2002-2005). We used regression models to examine associations of adiponectin, leptin, and resistin levels with (1) high-attenuation areas (HAAs) from CT scans (2004-2005, n = 1,144), (2) interstitial lung abnormalities (ILAs) from CT scans (2010-2012, n = 872), and (3) FVC from spirometry (2004-2006, n = 1,446). We used $-(1/HAA^2)$, which we denoted with *H*, to model HAA as our outcome to meet model assumptions.

RESULTS: Higher adiponectin was associated with lower HAA on CT imaging among adults with a BMI $\geq$ 25 kg/m^2 (*P* for BMI interaction = .07). Leptin was more strongly associated with ILA among never smokers compared with ever smokers (*P* for smoking interaction = .004). For every 1-SD increment of log-transformed leptin, the percent predicted FVC was 3.8% lower (95% CI, −5.0 to −2.5). Higher serum resistin levels were associated with greater HAA on CT in a fully adjusted model. For every 1-SD increment of log-transformed resistin there was an increase in *H* of 14.8 (95% CI, 3.4-26.3).

CONCLUSIONS: Higher adiponectin levels were associated with lower HAA on CT imaging among adults with a higher BMI. Higher leptin and resistin levels were associated with lower FVC and greater HAA, respectively. CHEST 2020; 157(3):580-589

KEY WORDS: adipokine; chest imaging; epidemiology (pulmonary); interstitial lung disease

ABBREVIATIONS: BALF = BAL fluid; HAA = high-attenuation areas; IL = interleukin; ILA = interstitial lung abnormality; ILD = interstitial lung disease; MESA = Multi-Ethnic Study of Atherosclerosis

AFFILIATIONS: From the Department of Medicine (Dr Kim), University of Virginia School of Medicine, Charlottesville, VA; the Department of Medicine (Drs Anderson, Podolanczuk, Barr, Lederer, and Giles), Columbia University Medical Center, New York, NY; the Department of Medicine and the Center for Clinical Epidemiology and Biostatistics (Dr Kawut), Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA; the Department of Family and Preventative Medicine (Dr Allison), University of California San Diego, San Diego, CA; the Department of Medicine (Dr Raghu), University of Washington, Seattle, WA; the Department of Biostatistics (Ms Hinckley-Stuckovsky), University of Washington, Seattle, WA; the Departments of Radiology, Medicine, and Biomedical Engineering (Dr Hoffman), University of Iowa Carver College of Medicine, Iowa City, IA; the Departments of Pathology and Laboratory Medicine (Dr Tracy), University of Vermont, Burlington, VT;

Adiposity (ie, total body fat amount and distribution) plays a significant role in many chronic diseases mediated by the production of adipose-derived hormones, termed "adipokines." Adipokines, such as adiponectin, leptin, and resistin, circulate systemically and participate in energy metabolism and insulin sensitivity.[1] They have emerging roles in immune function and fibrogenesis in various organs.[1] Adiponectin inhibits the proinflammatory transcription factor nuclear factor-κB (NF-κB) and increases production of the antiinflammatory cytokine interleukin (IL)-10.[2,3] In contrast, leptin and resistin are proinflammatory and increase production of helper T-cell type 1 cytokines and IL-1β, and stimulate monocyte and $CD4^+$ T-lymphocyte proliferation.[4-6] Signaling receptors of adipokines are expressed in alveolar type II and bronchial epithelial cells, which suggests that the lung is a target for adipokine signaling.[7-10]

Idiopathic pulmonary fibrosis is one of the most common types of interstitial lung disease (ILD), with treatments that slow but do not stop disease progression.[11-13] Therefore, the identification of modifiable risk factors (eg, adiposity) in adults with evidence of early lung inflammation and scarring, who may otherwise have minimal or no symptoms (ie, subclinical ILD), may lead to novel therapeutic targets.[14] High-attenuation areas (HAAs) and interstitial lung abnormalities (ILAs) are CT imaging-based measures of subclinical ILD and are associated with cough, dyspnea, overall mortality, and hospitalization and death due to ILD. HAAs and ILAs have been used to identify potential factors that may contribute to the early pathogenesis of ILD.[15-20]

Using the Multi-Ethnic Study of Atherosclerosis (MESA) cohort, we examined cross-sectional associations of serum adipokine levels with HAAs and ILAs from CT scans and FVC among community-dwelling adults.[21,22] We hypothesized that lower serum levels of adiponectin and higher levels of leptin and resistin would be associated with a greater proportion of HAAs and a higher prevalence of ILAs on CT imaging, and lower FVC. We performed stratified analyses to determine differences in the associations of adipokines with ILD measures according to previously identified factors related to adipokine production and chronic inflammatory diseases: BMI, sex, and smoking status.[23-27]

Methods

Study Participants

We used data from MESA, a National Heart, Lung, and Blood Institute-funded longitudinal cohort of community-dwelling adults.[22] MESA recruited 6,814 adults between the ages of 45 and 84 years without known clinical cardiovascular disease at the time of enrollment from six United States-based communities. Participants attended their first examination (exam) visit in 2000-2002 and underwent follow-up visits. As part of the MESA Body Composition ancillary study, a subset of randomly selected participants (n = 1,968) underwent serum adipokine measurement once during their follow-up visits between 2002 and 2005.[26] Of these participants, 1,967, 1,960, and 1,966 had valid adiponectin, leptin, and resistin measurements, respectively (e-Fig 1). Institutional review board approval was obtained at all sites (e-Table 1), and all participants provided written informed consent.

Measurements

Adiponectin, leptin, and resistin were measured from banked fasting serum samples collected between 2002 and 2005, using Bio-Rad Luminex flow cytometry (Millipore).[26] The Laboratory for Clinical Biochemistry Research (University of Vermont) conducted these measurements. Average analytic coefficients of variation were 5.8% for adiponectin, 5.2% for leptin, and 6.7% for resistin across several control samples.[26]

HAAs and ILAs were measured from exam 3 (2004-2005) noncontrast cardiac and exam 5 (2010-2012) full lung CT scans, respectively.[16] The mean (SD) time between exam 3 and exam 5 CT scans was 6.9 (0.9) years. HAA was defined as the percentage of voxels that have attenuation values between −600 and −250 Hounsfield units.[28] Using the Pulmonary Analysis Software Suite (PASS) at the University of Iowa's Advanced Pulmonary Physiomic Imaging Laboratory (APPIL), lungs were semiautomatically segmented and corrected by a trained technician.[29]

The MESA Lung/SPIROMICS protocol was used to perform full lung CT scans at exam 5.[30] ILA was defined as the presence of reticular abnormalities, ground-glass abnormalities, diffuse centrilobular nodularity, traction bronchiectasis, nonemphysematous cysts, and/or honeycombing with at least 5% of nondependent lung

and the Department of Epidemiology (Drs Barr and Lederer), Mailman School of Public Health, Columbia University Medical Center, New York, NY.

Drs Lederer and Giles contributed equally to this manuscript.

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CORRESPONDENCE TO: John S. Kim, MD, Division of Pulmonary and Critical Care Medicine, Department of Medicine, University of Virginia School of Medicine, P.O. Box 800546, Clinical Department Wing, 1 Hospital Dr, Charlottesville, VA 22908; e-mail: jk6jb@hscmail.mcc.virginia.edu

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involvement.[15] ILA was determined by one of five trained radiologists.[16] ILA was fully assessed only in exam 5 CT scans.

We used the results of spirometry that was performed approximately when serum was collected and used for adipokine measurements. Spirometry was performed at exams 3 and 4 (2004-2006) as part of the MESA Lung ancillary study and in accordance with the American Thoracic Society/European Respiratory Society guidelines.[31,32]

Statistical Analysis

We used linear regression models to examine cross-sectional associations of serum adipokine levels with HAAs from exam 3 CT scans and FVC from exam 3 or 4 spirometry. Logistic regression was used to examine associations of adipokine levels with ILAs from exam 5 CT scans. Directed acyclic graphs were used to conceptualize the potential causal pathway between adipokines and our outcomes of interest and covariates for our regression models (e-Fig 2).[33,34] On the basis of prior studies, we identified additional covariates that may influence adipokine levels, inflammation, and scarring, and included them in our model.[15,35-37] Model 1 was minimally adjusted for study site, imaged lung volume, and milliampere radiation dose in HAA analyses a priori.[16] For our FVC and ILA analyses, model 1 was unadjusted. Model 2 was additionally adjusted for age, sex, race/ethnicity, smoking status, missing smoking status indicator, cigarette pack-years, and statin medication use. Percent emphysema was also adjusted in our HAA and ILA analyses. Model 3 was adjusted for model 2 variables and BMI (kg/m^2). Generalized additive models were used to assess the linearity of associations between adipokine levels and our outcomes. Adipokine levels were log-transformed to fulfill assumptions for our regression models because of their right-skewed distribution (kurtosis $>$ 8.0).

Likelihood ratio tests were used to test for effect modification by BMI, sex, and smoking status in prespecified stratified analyses. We used a BMI cutoff of $\geq$ 25 kg/m^2 based on prior studies and guidelines that suggest adults above this cutoff are at higher risk for chronic diseases and death.[38,39] We assessed three-way interactions of BMI, sex, and smoking status on the associations between serum adipokine levels and our outcomes of interest. Details of how we assessed three-way interactions are described in e-Appendix 1. We used –(1/HAA^2), which we have termed "eta" (*H*), to model HAA as our outcome to meet model assumptions.

There were a small number of participants with missing cigarette pack-years data: HAA (n = 12), ILA (n = 10), and FVC (n = 15) (e-Fig 1). We used a missing cigarette pack-years indicator variable in our regression models. Statin medication use and diagnosis of diabetes were self-reported in MESA.[22] Twenty-five participants were missing statin medication data and they were excluded from the fully adjusted analyses for HAA, ILA, and FVC. All other covariates were complete. Results are reported per 1-SD increment of the natural log-transformed serum adipokine level. We used Stata 15.1 (StataCorp) and the "gam" package in R version 3.4.3 (R Foundation for Statistical Computing) for our analyses.

Results

Baseline Characteristics

Among those participants with a valid measurement for at least one type of serum adipokine, 1,144 underwent cardiac CT scans at exam 3, 1,446 participants performed spirometry at exams 3 or 4 (2004-2006), and 872 underwent full lung CT scans at exam 5 (2010-2012).[16] Overall baseline characteristics of the participants with available adipokine and HAA measurements are summarized in Table 1. Characteristics by serum adipokine quartiles are summarized in e-Tables 2-4.

CT Imaging-Based Subclinical ILD Measurements and Lung Function

Associations of serum adipokines with HAA, ILA, and FVC are summarized in Table 2. In a minimally adjusted model (model 1), higher serum adiponectin levels were associated with less HAA on CT imaging. After adjusting for baseline covariates (model 2), this association remained present. For every 1-SD increment of log-transformed adiponectin, there was a lower *H* of 23.7 units (95% CI, −36.7 to −10.7). After adjusting for BMI, this association was significantly attenuated (model 3). Higher serum leptin levels were positively associated with HAA on exam 3 CT imaging after adjusting for baseline covariates (model 2), but was negatively associated after adjusting for BMI (Table 2).

Higher levels of serum resistin were associated with greater HAA on CT imaging, even after adjusting for baseline covariates and BMI (model 3). For every 1-SD increment of log-transformed resistin there was a higher *H* of 14.8 units (95% CI, 3.4 to 26.3) (Fig 1, Table 2).

We did not detect an association between serum adipokine levels and ILA measured on full lung CT at exam 5 in the overall cohort (Table 2).

Serum adiponectin levels were not associated with percent predicted FVC. Higher serum levels of leptin and resistin were associated with lower percent predicted FVC measured at exams 3 and 4, even after adjusting for all covariates (model 3). For every 1-SD increment of log-transformed leptin and resistin, percent predicted FVC was 3.8% lower (95% CI, −5.0 to −2.5) and 0.9% lower (95% CI, −1.7 to −0.02), respectively (Fig 1, Table 2).

Stratified Analyses by BMI, Sex, and Smoking Status

Adiponectin levels were more strongly associated with HAAs among those with a BMI $\geq$ 25 kg/m^2 compared with those with a BMI $<$ 25 kg/m^2 (P = .07 for BMI interaction) (Fig 2). For every 1-SD increment of log-transformed adiponectin, *H* was 21.9 units lower (95% CI, −36.9 to −6.9) among those with a BMI $\geq$ 25 kg/m^2 (e-Table 5). We did not detect evidence that

TABLE 1] Baseline Characteristics

Characteristic	Value
No. of participants with HAAs on exam 3 CT imaging	1,144
Serum adipokine, median (IQR)	
Adiponectin, ng/mL	17,588 (15,163)
Leptin, pg/mL	13,124 (22,780)
Resistin, pg/mL	14,734 (6,678)
Female	50%
Age, y	64 (9)
Race/ethnicity	
White	49%
Asian	11%
African American	17%
Hispanic	23%
Weight, kg	78 (17)
Height, cm	167 (10)
BMI, kg/m^2	28 (5)
Waist circumference, cm	98 (14)
Statin medication use	23%
Diabetes	10%
Smoking status	
Never smoker	45%
Former smoker	44%
Current smoker	11%

Serum adipokines are presented as median (IQR). All other data are presented as mean (SD) for continuous variables and as percentages for categorical variables. Serum adipokines were measured between 2002 and 2005. HAAs were measured from exam 3 CT scans (2004-2005). HAA = high-attenuation area; IQR = interquartile range.

BMI modified associations of leptin and resistin with HAA, ILA, or percent predicted FVC (e-Table 5).

Associations of serum leptin and resistin with percent predicted FVC were stronger among men compared with women (all P values for sex interaction $\leq$ .007). The percent predicted FVC was lower by 4.5% (95% CI, −5.9 to −3.2) among men compared with 1.7% (95% CI, −3.6 to 0.2) among women per 1-SD increment of log-transformed leptin (Fig 3, e-Table 6). For every 1-SD increment of log-transformed resistin, the percent predicted FVC was lower by 2.0% (95% CI, −3.2 to −0.8) among men compared with a higher percent predicted FVC of 0.3% (95% CI, −0.9 to 1.4) among women. There was no effect modification by sex on the associations of serum adipokine levels with HAA or ILA.

Higher leptin levels were more strongly associated with less HAA on CT among ever smokers compared with never smokers (P = .002 for smoking interaction) (e-Table 7). Higher serum leptin levels were more strongly associated with ILA among never smokers compared with ever smokers (P = .004 for smoking interaction) (Fig 4, e-Table 7). For every 1-SD increment of log-transformed leptin, there were 100% higher odds of having ILA (95% CI, 1.2-3.3).

The association between serum adiponectin and HAA was strongest among women with a BMI $\geq$ 25 kg/m^2 (P = .005 for BMI and sex interaction), such that for every 1-SD increment of log-transformed adiponectin, there was a lower H of 39.2 units (95% CI, −60.3 to −18.0) (e-Table 8). The association between leptin and HAA was strongest among never smokers with a BMI $\geq$ 25 kg/m^2 (P = .02 for BMI and smoking interaction). Full results of these three-way interactions are presented in e-Tables 8 and 9.

Discussion

In our study, we found that higher serum levels of resistin were associated with greater HAA and lower FVC. Higher leptin levels were associated with lower FVC overall and a greater prevalence of ILA on CT imaging among never smokers. Adiponectin was associated with less HAA on CT imaging among those with a BMI $\geq$ 25 kg/m^2. Our findings suggest that higher circulating levels of proinflammatory adipokines, and lower levels of adiponectin, are associated with greater subclinical lung inflammation and scarring on CT imaging and lower lung function.

The role of body habitus in ILD remains unclear. Lower baseline BMI and a decline in BMI over time among adults with pulmonary fibrosis were each associated with a higher overall mortality risk.[40,41] Circulating adipokines may play a role as higher circulating levels of leptin are associated with a higher risk of acute exacerbations of pulmonary fibrosis.[42] Leptin promotes the expression and activity of transforming growth factor-β_1 via inhibition of peroxisome proliferator-activated receptor-γ in lung fibrosis mouse models.[43] Leptin accelerates pulmonary fibrosis by inhibiting autophagy through activation of the phosphatidylinositol 3-kinase (PI3K)/Akt/mammalian target of rapamycin (mTOR) pathway.[44] Our findings support this as higher leptin levels were associated with lower FVC overall and higher odds of ILA on CT imaging among never smokers in MESA. The positive association between leptin and HAA (model 2) was reversed after adjusting for BMI (model 3) and suggests

TABLE 2] Associations of Serum Adipokine With High-Attenuation Areas and Interstitial Lung Abnormalities on CT Imaging and FVC

Serum Adipokine	No.	Change in *H* Per 1-SD Increment of Log-Transformed Adipokine	95% CI	No.	Odds of ILA Per 1-SD Increment of Log-Transformed Adipokine	95% CI	No.	Change in % Predicted FVC Per 1-SD Increment of Log-Transformed Adipokine	95% CI
Adiponectin									
Model 1	1,144	−37.1	−50.9 to −23.3	872	1.2	0.96 to 1.5	1,446	0.5%	−0.3 to 1.4
Model 2	1,120	−23.7	−36.7 to −10.7	852	1.1	0.9 to 1.4	1,421	1.1%	0.2 to 2.0
Model 3	1,120	−4.8	−17.6 to −7.9	852	1.1	0.9 to 1.4	1,421	0.3%	−0.7 to 1.2
Leptin									
Model 1	1,141	−0.7	−16.0 to 14.6	868	1.1	0.9 to 1.3	1,441	−3.6%	−4.4 to −2.8
Model 2	1,117	43.1	28.6 to 57.6	848	1.1	0.8 to 1.4	1,416	−4.5%	−5.5 to −3.5
Model 3	1,117	−11.9	−29.5 to 5.7	848	1.2	0.9 to 1.7	1,416	−3.8%	−5.0 to −2.5
Resistin									
Model 1	1,143	26.5	12.8 to 40.2	871	1.1	0.9 to 1.4	1,445	−1.0%	−1.9 to −0.2
Model 2	1,119	22.8	10.8 to 34.8	851	1.1	0.9 to 1.3	1,420	−1.1%	−2.0 to −0.3
Model 3	1,119	14.8	3.4 to 26.3	851	1.1	0.9 to 1.3	1,420	−0.9%	−1.7 to −0.02

Serum adipokines were measured between 2002 and 2005. High-attenuation areas (HAAs), percent predicted FVC, and interstitial lung abnormalities (ILAs) were measured at exam 3 (2004-2005), exams 3 or 4 (2004-2006), and exam 5 (2010-2012), respectively. Model 1 for HAA is a minimally adjusted model (exam 3 study site, radiation dose, and imaged lung volume). Model 1 is unadjusted for ILA and percent predicted FVC models. Model 2 is model 1 with additional adjustment for exam 3 age, sex, race/ethnicity, smoking status, cigarette pack-years, missing cigarette pack-year indicator, and statin medication use. Percent emphysema was also adjusted for in HAA and ILA models. Model 3 is model 2 with additional adjustment for BMI. $H = -(1/HAA^2)$.

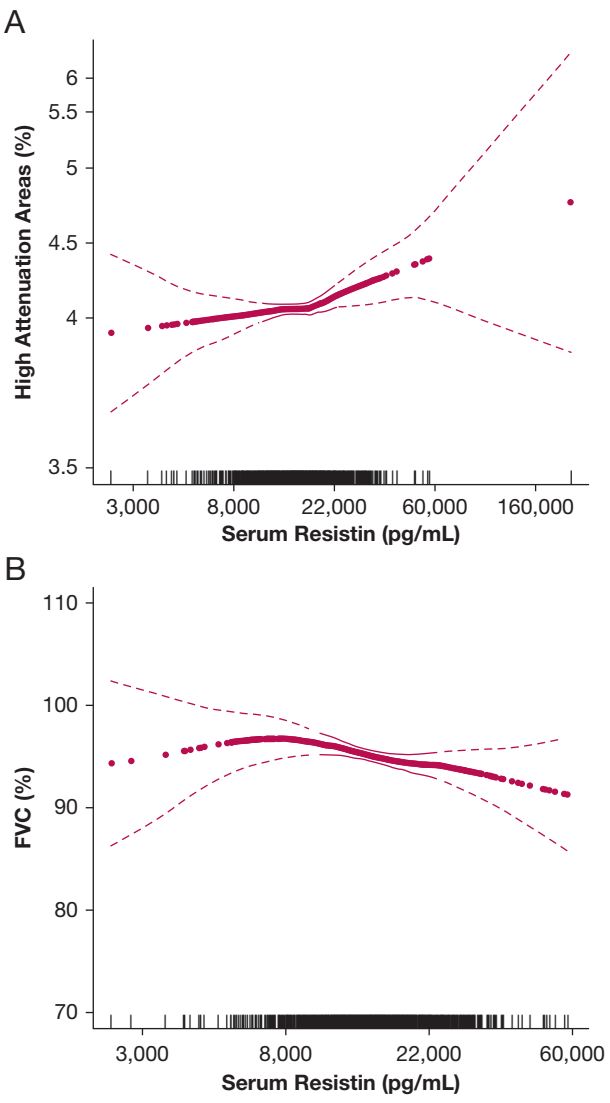

Figure 1 – *A and B, Continuous associations of serum resistin with high-attenuation areas (n = 1,119) (A) and % predicted FVC (n = 1,420) (B). A, Overall* P *for association = .01. B, Overall* P *for association = .045. Models are adjusted for exam 3 age, sex, race/ethnicity, smoking status, cigarette pack-years, missing cigarette pack-years indicator, statin medication use, and BMI. HAA model is also adjusted for study site, radiation dose, percent emphysema, and total lung volume imaged. The* x *axis is log-scale. The solid line is the overall effect estimate; thin dashed lines represent the 95% confidence interval bands. Each vertical hash-mark in the rug plot along the* x *axis represents one participant.*

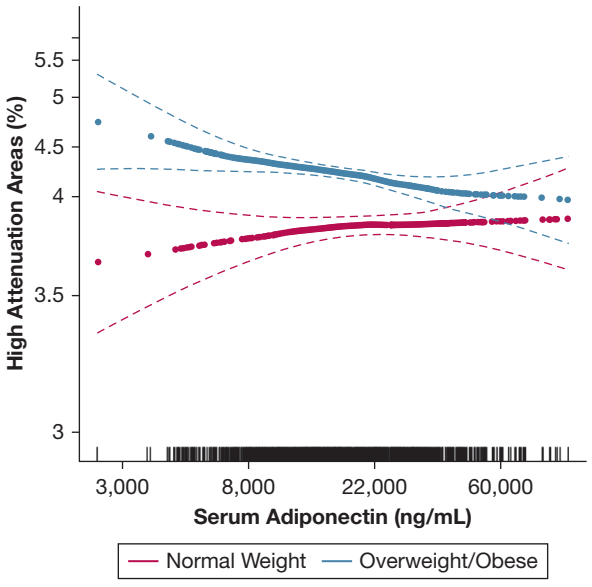

Figure 2 – *Continuous associations of serum adiponectin with high-attenuation areas stratified by BMI. Blue line: adults with BMI ≥ 25 kg/m^2 (n = 793). Red line: adults with BMI < 25 kg/m^2 (n = 327).* P *for BMI interaction = .07. Model is adjusted for exam 3 age, sex, race/ethnicity, smoking status, cigarette pack-years, missing cigarette pack-years indicator, statin medication use, percent emphysema, study site, radiation dose, and total lung volume imaged. The* x *axis is log-scale. The solid line represents the overall effect estimate; thin dashed lines indicate the 95% confidence interval bands. Each vertical hashmark in the rug plot along the* x *axis represents one participant.*

that body habitus is a strong confounder in the association between leptin and HAA. While HAA captures ILD-specific CT imaging characteristics (ie, reticulation, honeycombing), it may include BMI-related phenotypes such as atelectasis and increased attenuation from greater soft tissue.[45] Also, studies have proposed that higher circulating leptin levels reflect poor leptin receptor activation triggered by diet-induced obesity.[46] Therefore, leptin-induced inflammation may be blunted among adults with higher BMI. When we restricted our analysis to adults with a BMI < 25 kg/m^2, higher leptin was associated with greater HAA although the confidence intervals crossed zero. We caution that this interpretation is speculative and further investigation is needed.

Serum resistin had the most consistent association with subclinical ILD and lung function. Initially characterized as a mediator of insulin resistance and diabetes, resistin is critical in the cyclic AMP (cAMP)-mediated protein kinase A (PKA) activation and NF-κB-mediated transcription of inflammatory cytokines through its activation of adenylate cyclase-associated protein 1 (CAP1).[1,6] Resistin in humans is distinct in that adipose tissue monocytes and macrophages are the primary sources of this adipokine, signifying its immunomodulatory role.[47] Mice expressing humanized resistin on exposure to lipopolysaccharide demonstrated increased neutrophil extracellular trap formation and concentrations of histone 3, suggesting that Toll-like receptor-4 enhancement may be one of the mechanisms linking resistin to lung injury.[48] Resistin also induces mucin 5B (MUC5B) expression in human airway epithelial cells, and *MUC5B* gene promoter variants confer a significant risk for pulmonary fibrosis.[49,50]

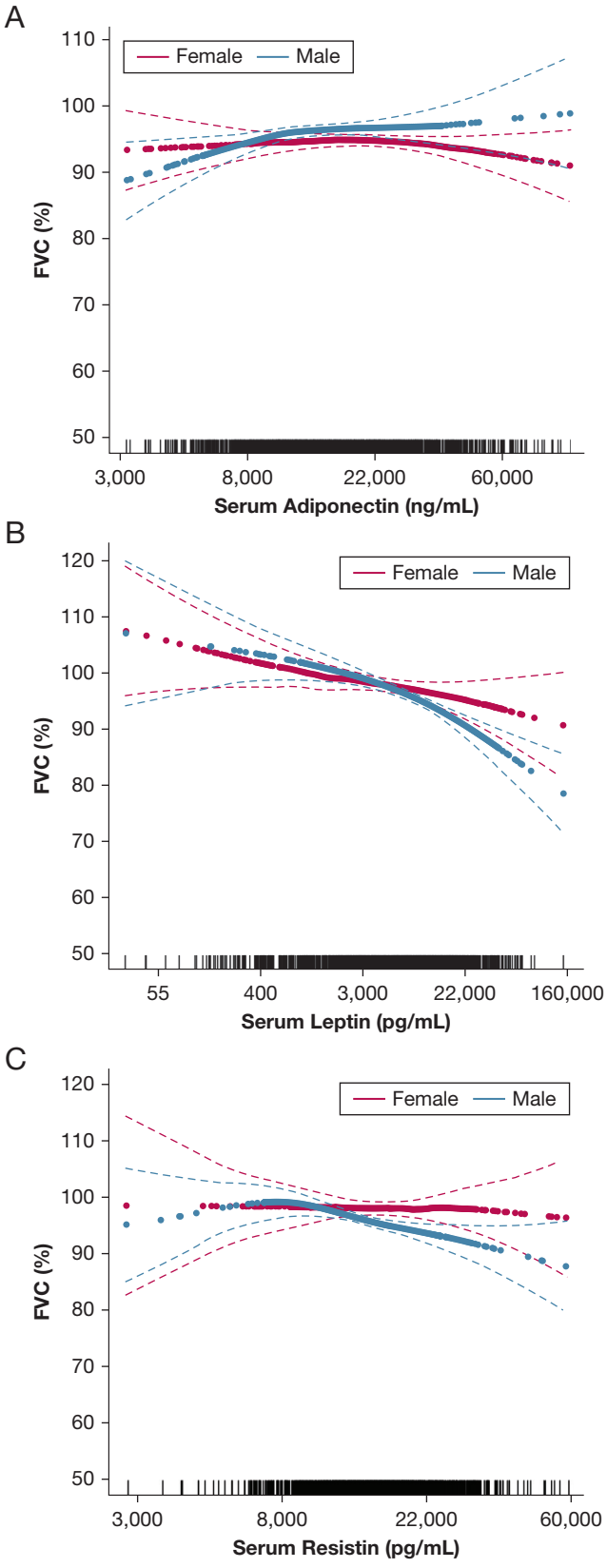

Figure 3 – *A-C, Continuous associations of serum adiponectin (A), leptin (B), and resistin (C) with percent predicted FVC stratified by sex. A, Red line, women (n = 681); blue line, men (n = 740);* P *for sex interaction = .06. B, Red line, women (n = 681); blue line, men (n = 735);* P *for sex interaction = .006. C, Red line, women (n = 681); blue line, men (n = 739);* P *for sex interaction = .007. Model is adjusted for exam 3 age, race/ethnicity, smoking status, cigarette pack-years, missing cigarette pack-years indicator, statin medication use, and BMI. The* x *axis is log-scale. The solid line represents the overall effect estimate; thin dashed lines indicate the 95% confidence interval bands. Each vertical hashmark in the rug plot along the* x *axis represents one participant.*

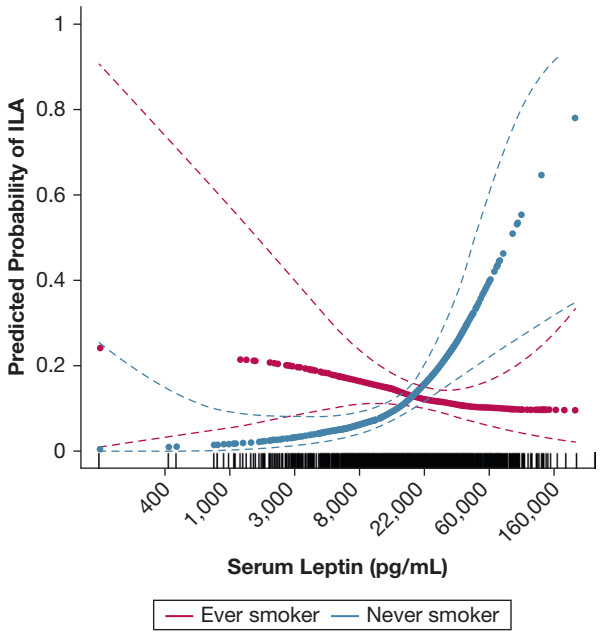

Figure 4 – *Continuous associations of serum leptin with interstitial lung abnormalities stratified by smoking status. Red line, ever smokers (n = 433); blue line, never smokers (n = 415).* P *for smoking interaction = .004. Model is adjusted for exam 3 age, sex, race/ethnicity, statin medication use, percent emphysema, and BMI. The* x *axis is log-scale. The solid line represents the overall effect estimate; thin dashed lines indicate the 95% confidence interval bands. Each vertical hashmark in the rug plot along the* x *axis represents one participant.*

BMI, sex, and smoking status are factors that may cause significant changes in the production of adipokines and modify their associations with diseases.[27] Those with higher BMI had a stronger inverse association between serum adiponectin and HAA on CT scan compared with those with a lower BMI. Overweight and obese individuals have lower circulating levels of adiponectin compared with normal weight adults, likely from local inhibition of adiponectin production in response to increased production of tumor necrosis factor-α and IL-6.[51] Adiponectin attenuated worsening lung fibrosis in paraquat-induced mouse models by suppressing lung fibroblast activation.[52] Individuals with lower circulating adiponectin may be more vulnerable to other mechanisms of repetitive lung injury (eg, acid reflux, infection, smoking), leading to recurrent injury and abnormal remodeling.

Men have lower circulating levels of adiponectin and leptin compared with women, likely due to regulatory effects of androgens.[23-25] Therefore, associations between circulating adipokines and lung function may be significantly different between women and men. We observed that associations of leptin and resistin with FVC were stronger among men compared with women and that the inverse association between adiponectin and HAA among overweight/obese adults was stronger in women than men.

Although leptin is considered a proinflammatory adipokine, studies suggest it is critical in a balanced immune response to cigarette smoke.[7,53] Mice deficient in leptin signaling that are exposed to cigarette smoke have higher numbers of neutrophils in their BAL fluid (BALF) and lung tissue compared with wild-type cigarette smoke-exposed mice, through increased expression of the neutrophil chemoattractant CXCL1.[53] More controlled neutrophil recruitment in the lung with cigarette exposure, due to greater leptin expression, may be why we observed a negative association of leptin with HAA among ever smokers. Although this finding was not replicated with ILA from exam 5 CT scans, leptin was associated with higher odds of ILA among never smokers after adjustment for covariates, including emphysema. This suggests that cigarette exposure modifies associations between leptin and subclinical ILD independent of emphysema. We caution against overinterpretation of these findings, due to the lack of mechanistic data.

Associations of serum adipokines with HAA overall and stratified analyses were more robust than for ILA. There are several explanations for this discrepancy. Although HAAs are associated with ILA, they are not predictive of ILA.[45] HAA was measured from CT scans performed closer in time to when the adipokines were measured, whereas ILA was assessed only once in MESA and nearly 10 years later. Circulating levels of adipokines may have changed significantly during the interval between HAA and ILA assessments, which may contribute to the overall null ILA findings. Repeat adipokine measurements were not performed in MESA at later examinations, as future studies examining longitudinal changes in adipokine measurements and HAA/ILA progression will be informative.

Our study has several limitations. We do not have repeat measurements of serum adipokines, as our analyses were cross-sectional, and reverse causation cannot be excluded. We caution against the drawing of causal inferences from this study as longitudinal studies with mechanistic data are needed. Adipokines were measured only in blood and not in lung tissue samples or BALF. BALF leptin levels correlate with circulating levels and suggest that serum adipokines are reasonable markers of lung expression and activity.[7] The sample size for our ILA analyses was notably smaller than for the HAA and FVC analyses, as fewer participants underwent exam 5

CT scans and ILA assessments. We cannot rule out that the smaller sample size limits the generalizability of our findings. Although we used directed acyclic graphs to build our regression models, unaccounted-for confounding factors remain a possibility.

In summary, our findings suggest that adiposity, through the production of adipokines, may play a role in the early pathogenesis of ILD. Future mechanistic studies will be informative and help identify those individuals who may be at higher risk of developing ILD.

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References

1. Mancuso P. The role of adipokines in chronic inflammation. *Immunotargets Ther*. 2016;5:47-56.
2. Ajuwon KM, Spurlock ME. Adiponectin inhibits LPS-induced NF-κB activation and IL-6 production and increases PPARγ2 expression in adipocytes. *Am J Physiol Regul Integr Comp Physiol*. 2005;288(5):R1220-R1225.
3. Kumada M, Kihara S, Ouchi N, et al. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation*. 2004;109(17):2046-2049.
4. Faggioni R, Jones-Carson J, Reed DA, et al. Leptin-deficient (*ob/ob*) mice are protected from T cell-mediated hepatotoxicity: role of tumor necrosis factor α and IL-18. *Proc Natl Acad Sci U S A*. 2000;97(5):2367-2372.
5. Gainsford T, Willson TA, Metcalf D, et al. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. *Proc Natl Acad Sci U S A*. 1996;93(25):14564-14568.
6. Lee S, Lee HC, Kwon YW, et al. Adenylyl cyclase-associated protein 1 is a receptor for human resistin and mediates inflammatory actions of human monocytes. *Cell Metab*. 2014;19(3):484-497.
7. Vernooy JH, Drummen NE, van Suylen RJ, et al. Enhanced pulmonary leptin expression in patients with severe COPD and asymptomatic smokers. *Thorax*. 2009;64(1):26-32.
8. Miller M, Cho JY, Pham A, Ramsdell J, Broide DH. Adiponectin and functional adiponectin receptor 1 are expressed by airway epithelial cells in chronic obstructive pulmonary disease. *J Immunol*. 2009;182(1):684-691.
9. Ali Assad N, Sood A. Leptin, adiponectin and pulmonary diseases. *Biochimie*. 2012;94(10):2180-2189.
10. Nigro E, Scudiero O, Sarnataro D, et al. Adiponectin affects lung epithelial A549 cell viability counteracting TNFα and IL-1β toxicity through AdipoR1. *Int J Biochem Cell Biol*. 2013;45(6):1145-1153.
11. King TE Jr, Bradford WZ, Castro-Bernardini S, et al. A phase 3 trial of pirfenidone in patients with idiopathic pulmonary fibrosis. *N Engl J Med*. 2014;370(22):2083-2092.
12. Richeldi L, du Bois RM, Raghu G, et al. Efficacy and safety of nintedanib in idiopathic pulmonary fibrosis. *N Engl J Med*. 2014;370(22):2071-2082.
13. Lederer DJ, Martinez FJ. Idiopathic pulmonary fibrosis. *N Engl J Med*. 2018;378(19):1811-1823.
14. Doyle TJ, Hunninghake GM, Rosas IO. Subclinical interstitial lung disease: why you should care. *Am J Respir Crit Care Med*. 2012;185(11):1147-1153.
15. Washko GR, Hunninghake GM, Fernandez IE, et al. Lung volumes and emphysema in smokers with interstitial lung abnormalities. *N Engl J Med*. 2011;364(10):897-906.
16. Podolanczuk AJ, Oelsner EC, Barr RG, et al. High attenuation areas on chest computed tomography in community-dwelling adults: the MESA study. *Eur Respir J*. 2016;48(5):1442-1452.
17. Podolanczuk AJ, Raghu G, Tsai MY, et al. Cholesterol, lipoproteins and subclinical interstitial lung disease: the MESA study. *Thorax*. 2017;72(5):472-474.
18. Sack C, Vedal S, Sheppard L, et al. Air pollution and subclinical interstitial lung disease: the Multi-Ethnic Study of Atherosclerosis (MESA) air-lung study. *Eur Respir J*. 2017;50(6):1700559.
19. Sack CS, Doney BC, Podolanczuk AJ, et al. Occupational exposures and subclinical interstitial lung disease. the MESA (Multi-Ethnic Study of Atherosclerosis) air and lung studies. *Am J Respir Crit Care Med*. 2017;196(8):1031-1039.
20. Kim JS, Podolanczuk AJ, Borker P, et al. Obstructive sleep apnea and subclinical interstitial lung disease in the Multi-Ethnic Study of Atherosclerosis (MESA). *Ann Am Thorac Soc*. 2017;14(12):1786-1795.
21. du Bois RM, Weycker D, Albera C, et al. Forced vital capacity in patients with idiopathic pulmonary fibrosis: test properties and minimal clinically important difference. *Am J Respir Crit Care Med*. 2011;184(12):1382-1389.
22. Bild DE, Bluemke DA, Burke GL, et al. Multi-Ethnic Study of Atherosclerosis: objectives and design. *Am J Epidemiol*. 2002;156(9):871-881.
23. Hickey MS, Israel RG, Gardiner SN, et al. Gender differences in serum leptin levels in humans. *Biochem Mol Med*. 1996;59(1):1-6.
24. Nishizawa H, Shimomura I, Kishida K, et al. Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. *Diabetes*. 2002;51(9):2734-2741.
25. Lanfranco F, Zitzmann M, Simoni M, Nieschlag E. Serum adiponectin levels in hypogonadal males: influence of testosterone replacement therapy. *Clin Endocrinol (Oxf)*. 2004;60(4):500-507.
26. Rasmussen-Torvik LJ, Wassel CL, Ding J, et al. Associations of body mass index and

insulin resistance with leptin, adiponectin, and the leptin-to-adiponectin ratio across ethnic groups: the Multi-Ethnic Study of Atherosclerosis (MESA). *Ann Epidemiol*. 2012;22(10):705-709.

27. Kotani K, Hazama A, Hagimoto A, et al. Adiponectin and smoking status: a systematic review. *J Atheroscler Thromb*. 2012;19(9):787-794.
28. Lederer DJ, Enright PL, Kawut SM, et al. Cigarette smoking is associated with subclinical parenchymal lung disease: the Multi-Ethnic Study of Atherosclerosis (MESA)-lung study. *Am J Respir Crit Care Med*. 2009;180(5):407-414.
29. Hoffman EA, Reinhardt JM, Sonka M, et al. Characterization of the interstitial lung diseases via density-based and texture-based analysis of computed tomography images of lung structure and function. *Acad Radiol*. 2003;10(10):1104-1118.
30. Sieren JP, Newell JD Jr, Barr RG, et al. SPIROMICS protocol for multicenter quantitative computed tomography to phenotype the lungs. *Am J Respir Crit Care Med*. 2016;194(7):794-806.
31. Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26(2):319-338.
32. Hankinson JL, Kawut SM, Shahar E, Smith LJ, Stukovsky KH, Barr RG. Performance of American Thoracic Society-recommended spirometry reference values in a multiethnic sample of adults: the Multi-Ethnic Study of Atherosclerosis (MESA) lung study. *Chest*. 2010;137(1):138-145.
33. Greenland S, Pearl J, Robins JM. Causal diagrams for epidemiologic research. *Epidemiology*. 1999;10(1):37-48.
34. Lederer DJ, Bell SC, Branson RD, et al. Control of confounding and reporting of results in causal inference studies. guidance for authors from editors of respiratory, sleep, and critical care journals. *Ann Am Thorac Soc*. 2019;16(1):22-28.
35. Barr RG, Bluemke DA, Ahmed FS, et al. Percent emphysema, airflow obstruction, and impaired left ventricular filling. *N Engl J Med*. 2010;362(3):217-227.
36. Chrusciel P, Sahebkar A, Rembek-Wieliczko M, et al. Impact of statin therapy on plasma adiponectin concentrations: a systematic review and meta-analysis of 43 randomized controlled trial arms. *Atherosclerosis*. 2016;253:194-208.
37. Kreuter M, Bonella F, Maher TM, et al. Effect of statins on disease-related outcomes in patients with idiopathic pulmonary fibrosis. *Thorax*. 2017;72(2):148-153.
38. Bhaskaran K, Dos-Santos-Silva I, Leon DA, Douglas IJ, Smeeth L. Association of BMI with overall and cause-specific mortality: a population-based cohort study of 3.6 million adults in the UK. *Lancet Diabetes Endocrinol*. 2018;6(12):944-953.
39. Expert Panel on the Identification, Evaluation, and Treatment of Overweight in Adults. Clinical guidelines on the identification, evaluation, and treatment of overweight and obesity in adults: executive summary. *Am J Clin Nutr*. 1998;68(4):899-917.
40. Alakhras M, Decker PA, Nadrous HF, Collazo-Clavell M, Ryu JH. Body mass index and mortality in patients with idiopathic pulmonary fibrosis. *Chest*. 2007;131(5):1448-1453.
41. Pugashetti J, Graham J, Boctor N, et al. Weight loss as a predictor of mortality in patients with interstitial lung disease. *Eur Respir J*. 2018;52(3):1801289.
42. Cao M, Swigris JJ, Wang X, et al. Plasma leptin is elevated in acute exacerbation of idiopathic pulmonary fibrosis. *Mediators Inflamm*. 2016;2016:6940480.
43. Jain M, Budinger GR, Lo A, et al. Leptin promotes fibroproliferative acute respiratory distress syndrome by inhibiting peroxisome proliferator-activated receptor-γ. *Am J Respir Crit Care Med*. 2011;183(11):1490-1498.
44. Gui X, Chen H, Cai H, Sun L, Gu L. Leptin promotes pulmonary fibrosis development by inhibiting autophagy via PI3K/Akt/mTOR pathway. *Biochem Biophys Res Commun*. 2018;498(3):660-666.
45. Kliment CR, Araki T, Doyle TJ, et al. A comparison of visual and quantitative methods to identify interstitial lung abnormalities. *BMC Pulm Med*. 2015;15:134.
46. Myers MG Jr, Leibel RL, Seeley RJ, Schwartz MW. Obesity and leptin resistance: distinguishing cause from effect. *Trends Endocrinol Metab*. 2010;21(11):643-651.
47. Qatanani M, Szwergold NR, Greaves DR, Ahima RS, Lazar MA. Macrophage-derived human resistin exacerbates adipose tissue inflammation and insulin resistance in mice. *J Clin Invest*. 2009;119(3):531-539.
48. Jiang S, Park DW, Tadie JM, et al. Human resistin promotes neutrophil proinflammatory activation and neutrophil extracellular trap formation and increases severity of acute lung injury. *J Immunol*. 2014;192(10):4795-4803.
49. Kwak S, Kim YD, Na HG, Bae CH, Song SY, Choi YS. Resistin upregulates *MUC5AC/B* mucin gene expression in human airway epithelial cells. *Biochem Biophys Res Commun*. 2018;499(3):655-661.
50. Seibold MA, Wise AL, Speer MC, et al. A common *MUC5B* promoter polymorphism and pulmonary fibrosis. *N Engl J Med*. 2011;364(16):1503-1512.
51. Bruun JM, Lihn AS, Verdich C, et al. Regulation of adiponectin by adipose tissue-derived cytokines: in vivo and in vitro investigations in humans. *Am J Physiol Endocrinol Metab*. 2003;285(3):E527-E533.
52. Yao R, Cao Y, He YR, Lau WB, Zeng Z, Liang ZA. Adiponectin attenuates lung fibroblasts activation and pulmonary fibrosis induced by paraquat. *PLoS One*. 2015;10(5):e0125169.
53. Vernooy JH, Bracke KR, Drummen NE, et al. Leptin modulates innate and adaptive immune cell recruitment after cigarette smoke exposure in mice. *J Immunol*. 2010;184(12):7169-7177.