Impaired lung function and serum leptin in men and women with normal body weight: a population-based study

D D Sin, S F P Man

Background: Impaired lung function is a risk factor for cardiovascular morbidity. Whether circulating factors are responsible for this association is unknown. A study was undertaken to determine whether leptin, a hormone that can promote atherothrombosis, is raised in individuals with impaired lung function.

Methods: Data from non-obese participants in the Third National Health, Nutrition, and Examination Survey (n=2808) were analysed to determine the relationship between circulating leptin levels and forced expiratory volume in 1 second (FEV₁) values divided into quintiles (quintile 1, FEV₁ predicted ≤85.2%; quintile 2, 85.3–94.3%; quintile 3, 94.4–101.4%; quintile 4, 101.5–110.0%; and quintile 5, ≥110.1%).

Results: Serum leptin levels changed along the FEV₁ gradient. The highest leptin levels were found in quintile 1 (geometric mean (GM) 5.42; interquartile range (IQR) 3.00–9.60 fg/l) and the lowest in quintile 5 (GM 4.94, IQR 2.80–9.10 fg/l). Adjustments for age, body mass index, and other confounders strengthened this relationship. Compared with quintile 5, the odds of having an increased serum leptin level in quintiles 1, 2, 3, and 4 were 2.26 (95% confidence interval (CI) 1.54 to 3.31), 2.20 (95% CI 1.52 to 3.17), 1.46 (95% CI 1.01 to 2.09), and 1.28 (95% CI 0.90 to 1.83), respectively.

Conclusion: Individuals with impaired lung function have raised serum leptin levels. Leptin may play a role in the pathogenesis of cardiovascular morbidity and mortality related to impaired lung function.

There is a growing body of evidence to indicate that reduced forced expiratory volume in 1 second (FEV₁) is a major risk factor for cardiovascular morbidity and mortality.1-5 In one study6 FEV₁, was shown to be a better predictor of cardiovascular mortality than some traditional risk factors such as total serum cholesterol. The overall strength and consistency of the relationship between FEV₁ and cardiovascular diseases among individuals with impaired lung function.

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provided a morning blood sample after an overnight fast. The minimum detectable concentration of this assay was 0.5 fg/l and the limit of linearity was up to 100 fg/l. Recovery of leptin was estimated using pack year equivalents (average daily smoking) and the limit of linearity was up to 100 fg/l. Recovery of leptin added to serum was 99–104% over the linear range of the assay. Other measurements (C reactive protein (CRP), fibrinogen, serum albumin, serum folate, and total cholesterol levels) were obtained using standard laboratory techniques. Covariates

Age was classified into six strata (20–29; 30–39; 40–49; 50–59; 60–69; and 70 years and older), race was divided into two categories (white and non-white), and smoking status was divided into three strata (current, former, and never smokers). For current and former smokers the total cigarette consumption was estimated using pack year equivalents (average daily consumption of cigarettes divided by 20 and multiplied by the number of years smoked). BMI was calculated using standard equations and expressed in kg/m²; it was divided into four quartiles (quartile 1, <21.20 kg/m²; quartile 2, 21.20–22.94 kg/m²; quartile 3, 22.95–24.49 kg/m²; and quartile 4, 24.50–25.90 kg/m²).

Statistical analyses

The baseline characteristics of the study participants across the FEV1 quintile groups were compared using a χ² test for binary variables and a t test for continuous variables. To assess whether there was a gradient in various baseline demographic and clinical factors across the lung function categories we used a Mantel-Haenszel test for trend. Multiple linear regression techniques were used to determine the independent relationship between FEV1 groups and serum leptin levels. Because serum leptin values were non-normally distributed, all serum leptin levels were log transformed for analytical purposes. In the final model we included BMI (in quartiles as well as a continuous variable), sex, age (in categories as well as a continuous variable), race, and smoking status (in pack years and in categories) as covariates. All tests were two tailed in nature and p values <0.05 were considered statistically significant. Continuous variables are shown as mean (SD) unless otherwise indicated, and serum leptin values are expressed as geometric mean (interquartile range) unless otherwise indicated.

Covariates

were defined as leptin values within the top 5th percentile. “Very raised” values as those within the top 25th percentile. “Very raised” levels were defined as leptin values within the top 5th percentile. Multiple logistic regression techniques (using the same covariates as described for linear regression models) were used to determine the impact of FEV1 groups on the risk of having raised or very raised serum leptin values. In all analyses quintile 5 was used as the referent group.

### Table 1 Baseline characteristics of study participants stratified according to severity of FEV1 impairment

<table>
<thead>
<tr>
<th>Severity of impairment</th>
<th>Quintile 1 (n=561)</th>
<th>Quintile 2 (n=562)</th>
<th>Quintile 3 (n=561)</th>
<th>Quintile 4 (n=562)</th>
<th>Quintile 5 (n=562)</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV1 (% predicted)</td>
<td>70.8 (14.9)</td>
<td>90.1 (2.6)</td>
<td>98.0 (2.1)</td>
<td>100.5 (2.4)</td>
<td>118.5 (8.3)</td>
<td>0.001</td>
</tr>
<tr>
<td>FEV1 (l)</td>
<td>2.15 (0.82)</td>
<td>2.95 (0.70)</td>
<td>3.23 (0.77)</td>
<td>3.46 (0.82)</td>
<td>3.53 (1.01)</td>
<td>0.001</td>
</tr>
<tr>
<td>FEV1/FVC (%)</td>
<td>80.7 (7.8)</td>
<td>82.3 (7.8)</td>
<td>83.9 (7.8)</td>
<td>82.3 (7.3)</td>
<td>82.3 (7.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Age (years)</td>
<td>53.1 (19.8)</td>
<td>42.8 (18.0)</td>
<td>40.2 (17.9)</td>
<td>39.7 (17.6)</td>
<td>46.3 (21.2)</td>
<td>0.001</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>22.5 (2.4)</td>
<td>22.6 (2.2)</td>
<td>22.6 (2.2)</td>
<td>22.9 (2.2)</td>
<td>22.9 (2.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Males</td>
<td>69.0 (14.3)</td>
<td>78.5 (8.3)</td>
<td>80.7 (7.8)</td>
<td>82.3 (7.0)</td>
<td>82.3 (7.0)</td>
<td>0.001</td>
</tr>
<tr>
<td>Whites</td>
<td>433 (77.2%)</td>
<td>422 (75.1%)</td>
<td>423 (75.4%)</td>
<td>426 (75.8%)</td>
<td>381 (67.8%)</td>
<td>0.002</td>
</tr>
<tr>
<td>Current smokers</td>
<td>227 (40.4%)</td>
<td>187 (33.3%)</td>
<td>151 (26.9%)</td>
<td>130 (23.1%)</td>
<td>136 (24.2%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Never smokers</td>
<td>213 (38.0%)</td>
<td>275 (48.9%)</td>
<td>301 (53.7%)</td>
<td>324 (57.7%)</td>
<td>321 (57.2%)</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum albumin (g/l)</td>
<td>40.9 (3.4)</td>
<td>41.6 (3.5)</td>
<td>41.6 (3.6)</td>
<td>41.9 (3.6)</td>
<td>41.6 (3.5)</td>
<td>0.001</td>
</tr>
<tr>
<td>Serum folate (mmol/l)</td>
<td>15.4 (14.2)</td>
<td>14.8 (14.7)</td>
<td>15.6 (15.6)</td>
<td>14.1 (12.3)</td>
<td>13.8 (12.8)</td>
<td>0.952</td>
</tr>
<tr>
<td>Total serum cholesterol (mmol/l)</td>
<td>5.3 (1.1)</td>
<td>5.1 (1.1)</td>
<td>5.0 (1.1)</td>
<td>5.0 (1.0)</td>
<td>5.2 (1.1)</td>
<td>0.154</td>
</tr>
<tr>
<td>CRP (mg/l)*</td>
<td>3.07</td>
<td>2.70</td>
<td>2.56</td>
<td>2.53</td>
<td>2.52</td>
<td>0.001</td>
</tr>
<tr>
<td>Leucocytes (&lt;10³/µl)</td>
<td>7.07 (2.41)</td>
<td>6.64 (2.09)</td>
<td>6.35 (1.96)</td>
<td>6.34 (1.86)</td>
<td>6.26 (1.97)</td>
<td>0.001</td>
</tr>
<tr>
<td>Fibrinogen (g/l)</td>
<td>3.23 (0.88)</td>
<td>3.08 (0.86)</td>
<td>3.03 (0.92)</td>
<td>2.88 (0.82)</td>
<td>2.94 (0.74)</td>
<td>0.001</td>
</tr>
<tr>
<td>Triceps skinfold (mm)</td>
<td>13.8 (6.6)</td>
<td>14.7 (6.9)</td>
<td>14.8 (6.6)</td>
<td>14.3 (6.6)</td>
<td>13.2 (6.1)</td>
<td>0.068</td>
</tr>
</tbody>
</table>

Continuous variables are shown as mean (SD) and dichotomous variables are shown as number (% of column totals) unless otherwise indicated.

BMI=body mass index; FEV1=forced expiratory volume in 1 second; FVC=forced vital capacity; CRP=C reactive protein.

*Geometric mean.

### Table 2 Log transformed serum leptin values stratified according to severity of lung impairment

<table>
<thead>
<tr>
<th>Severity of impairment</th>
<th>Quintile 1 (n=561)</th>
<th>Quintile 2 (n=562)</th>
<th>Quintile 3 (n=561)</th>
<th>Quintile 4 (n=562)</th>
<th>Quintile 5 (n=562)</th>
<th>p for trend</th>
</tr>
</thead>
<tbody>
<tr>
<td>Log transformed leptin values (fg/l)</td>
<td>1.69 (0.78)</td>
<td>1.67 (0.81)</td>
<td>1.68 (0.78)</td>
<td>1.65 (0.80)</td>
<td>1.60 (0.79)</td>
<td>0.059</td>
</tr>
<tr>
<td>Unadjusted partial regression coefficient</td>
<td>0.093 (0.047)</td>
<td>0.069 (0.047)</td>
<td>0.085 (0.047)</td>
<td>0.0565 (0.047)</td>
<td>0.0 (reference)</td>
<td>0.095</td>
</tr>
<tr>
<td>Partial regression coefficient adjusted for sex</td>
<td>0.182 (0.034)</td>
<td>0.119 (0.034)</td>
<td>0.099 (0.034)</td>
<td>0.050 (0.034)</td>
<td>0.0 (reference)</td>
<td>0.001</td>
</tr>
<tr>
<td>Partial regression coefficient adjusted for sex and BMI</td>
<td>0.250 (0.029)</td>
<td>0.174 (0.029)</td>
<td>0.141 (0.029)</td>
<td>0.056 (0.029)</td>
<td>0.0 (reference)</td>
<td>0.001</td>
</tr>
<tr>
<td>Fully adjusted partial regression coefficient†</td>
<td>0.236 (0.029)</td>
<td>0.190 (0.029)</td>
<td>0.160 (0.029)</td>
<td>0.077 (0.029)</td>
<td>0.0 (reference)</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Partial regression coefficients were derived from linear regression models. Each cell represents the mean (SE) expected increase in serum leptin values (on a logarithmic scale, fg/l) for each quintile group relative to the reference category (quintile 5). Adjusted for age, sex, smoking status, race, body mass index, and lung function (see methods). BMI=body mass index.
RESULTS

Data were used from 2808 participants of NHANES 3 who met the inclusion and exclusion criteria of the study. Of these participants, 1335 (47.5%) were men, 2085 (74.3%) were white, and 831 (29.6%) were current smokers. The mean age and BMI of the participants were 44.4 (19.6) years and 22.7 (4.88) kg/m², respectively. The mean FEV₁, FVC, FEV₁ (% predicted) and FVC (% predicted) were 3.06 (0.97) l, 3.89 (0.97) l, 96.6 (17.8)%, and 99.1 (15.6)%, respectively. The baseline characteristics in the different lung function categories are summarised in table 1.

Individuals with impaired lung function (quintile 1) were more likely to be older and had lower BMI and serum albumin than those with good lung function (quintile 5). Markers of systemic inflammation including CRP, leucocytes, and fibrinogen were significantly higher in individuals with reduced lung function than in those with preserved lung function, suggesting a “severity dependent” association of systemic inflammation across the FEV₁ gradient.

The narrow range of BMI of our cohort allowed us to look for a relationship between FEV₁ and serum leptin levels. There was a strong inverse relationship between FEV₁ and serum leptin. Crudely, the serum concentration of leptin was 5.42 (3.00–9.60) fg/l for quintile 1, 5.29 (2.80–10.1) fg/l for quintile 2, 5.38 (3.00–9.50) fg/l for quintile 3, 5.23 (2.90–9.60) fg/l for quintile 4, and 4.94 (2.80–9.10) fg/l for quintile 5.

Multivariate adjustments for various factors including age, sex, race, and smoking status strengthened the inverse relationship between FEV₁ and serum leptin levels (table 2). The most influential confounding variables were sex and BMI. Age, race and smoking status, on the other hand, made little difference to the overall results. In the adjusted analysis, quintile 5 had the highest odds of having a raised serum leptin level (odds ratio (OR) 2.26, 95% confidence interval (CI) 1.54 to 3.31 compared with quintile 5), followed by quintile 2 (OR 2.20, 95% CI 1.52 to 3.17), quintile 3 (OR 1.46, 95% CI 1.01 to 2.09), and quintile 4 (OR 1.28, 95% CI 0.90 to 1.83; fig 1). A similar pattern was observed when the odds for having a “very raised” serum leptin level were considered (quintile 1, OR 2.20, 95% CI 1.17 to 4.13; quintile 2, OR 2.66, 95% CI 1.45 to 4.88; quintile 3, OR 1.91, 95% CI 1.02 to 3.56; quintile 4, OR 1.38, 95% CI 0.73 to 2.61).

Other factors that were significantly correlated with serum leptin concentrations are shown in table 3. As expected, BMI was a very powerful predictor of serum leptin levels and sex was also an important predictor. Independent of other risk factors, raised CRP levels (>2.1 mg/l) were associated with a marked increase in serum leptin levels. Unadjusted, the serum leptin concentration was 6.34 (3.40–11.90) fg/l in those with raised CRP levels compared with only 5.00 (2.80–9.00) fg/l in those with CRP levels <2.1 mg/l. Adjustments for a variety of factors (see methods) made little difference to these results. Those with raised CRP levels were more likely to have raised levels of serum leptin (OR 1.63, 95% CI 1.24 to 2.15). Circulating fibrinogen and leucocytes, other markers of systemic inflammation, were also associated with serum leptin levels (table 3).

DISCUSSION

The principal finding of this study was that, independent of other risk factors, FEV₁ was inversely related to circulating leptin levels among a representative sample of the US non-obese adult population. Furthermore, there was a significant association between serum leptin and a variety of other inflammatory markers such as CRP, leucocytes, and fibrinogen, suggesting a potential role of leptin in the inflammatory cascade associated with advanced lung disease.

There is a growing body of evidence indicating that leptin may have an important role in upregulating the inflammatory system. Recent evidence indicates that macrophages, when exposed to leptin, markedly increase production of proinflammatory mediators such as tumour necrosis factor (TNF)-α and interleukins 6 and 12. Interestingly, factors such as TNF-α also promote the expression and release of leptin from adipose tissue, indicating a positive feedback mechanism. Since leptin is a potent inflammatory promoter it may exacerbate existing lung and systemic inflammation, contributing to the poor clinical outcomes of patients with various lung diseases.
Leptin is also an important risk factor for coronary events. It has a pressor effect on blood vessels through increased activation of the sympathetic nervous system which is thought to mediate obesity-related hypertension. Because excess adrenergic activity has also been noted in advanced lung disease, our findings suggest that leptin may play an important role in increasing the risk for poor cardiovascular outcomes in such patients. Other adverse effects of leptin have been demonstrated including increased risk of thrombosis through its action on platelets and of osteoporosis through its action on the central hypothalamic pathways. The risks of cardiovascular morbidity and mortality are markedly increased in the presence of low FEV1. In view of the wide array of harmful cardiovascular and neurohumoral effects of leptin, its role in the pathogenesis of cardiovascular outcomes of lung disease need to be further explored.

Superficially, our findings appear to differ from those of previous reports. In one study serum leptin levels were found to be lower in those with advanced lung disease than in healthy controls, a finding that was difficult to understand since, in the same study, TFN-α levels (a positive feedback mediator for leptin) were much higher in those with lung disease than in the control group. This study, however, was small and the noise associated with the measurement of leptin could have confounded the findings. In a subsequent study by the same group of investigators serum leptin levels were found to be higher in patients with chronic obstructive pulmonary disease (COPD) than in controls, but only in a subgroup of patients who were non-cachectic and had similar BMI to the healthy controls, suggesting that BMI is a critical confounder of this relationship. Our study was designed to minimise the potential for confounding. In our analysis we excluded overweight and obese individuals in the NHANES 3 study. To control for any residual confounding by age, sex, and (in particular) weight we used multiple regression techniques in which age, sex, and BMI were considered as covariates. By doing so, we avoided the shortcomings of previous studies.

Our study has several limitations which need to be highlighted. Firstly, the cross sectional nature of the NHANES 3 data precludes any inferences regarding the temporal nature of the relationship between FEV1, impaired lung function and serum leptin levels. Secondly, there were insufficient numbers of patients with very severe lung disease, so the relationship between serum leptin levels and very severe lung disease remains unknown.

In summary, our findings indicate that, when adjusted for BMI, age, sex, and other potential confounders, patients with impaired FEV1 have higher serum leptin levels than normal controls in a “severity dependent” manner. The growing body of evidence demonstrating the important deleterious effects of circulating leptin on the neurohumoral and cardiovascular systems raises the possibility that this hormone may contribute to poor outcomes of patients with common lung diseases such as COPD. Future work is needed to confirm our findings and to determine whether interventions that reduce circulating leptin in lung disease can improve the health status and outcomes of these patients.

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REFERENCES

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