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

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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 (FEV_1) values divided into quintiles (quintile 1, FEV_1 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 FEV_1 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 (FEV_1) is a major risk factor for cardiovascular morbidity and mortality.[1–5] In one study[5] FEV_1 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 FEV_1 and cardiovascular diseases across many different studies[2–5] suggest that poor respiratory function may, indeed, have a causal role in the pathogenesis of cardiovascular conditions. The circulating factor(s) responsible for this process, however, remain largely unknown.

Increasingly, systemic inflammation is felt to be a critical centrepiece of atherothrombosis. Among the different hormones and cytokines that have been implicated in this process there is credible evidence that leptin, a hormone produced and secreted by adipose tissues normally in proportion to the amount of fat mass present, may be an important player in this cascade.[6] Leptin has been shown to upregulate systemic inflammation,[7] induce platelet aggregation,[8] and promote atherothrombosis.[9] These effects may be important in causing cardiovascular diseases among individuals with impaired lung function.

However, there is a paucity of population based data which have established a clear and unequivocal relationship between serum leptin and FEV_1. Data from previous studies[7 10 11] have been difficult to interpret due, in part, to various methodological limitations including small sampling and confounding by obesity and other factors. To address these shortcomings we analysed data from a large population based study, the Third National Health, Nutrition and Examination Survey (NHANES 3). Specifically, we sought to determine the independent relationship between FEV_1 (as percentage predicted) and circulating leptin concentrations in a representative sample of the US population. We hypothesised that circulating concentrations of leptin would be inversely related to FEV_1.

METHODS
Study participants
The detailed methods for NHANES 3 have been described previously.[12] Briefly, it comprised a cross sectional, multistage probability sample representative of the total non-institutionalised civilian population in the US. The sample was selected from households in 81 counties between 1988 and 1994. The original NHANES 3 sample included 20 050 adults aged 17 years and older. The present analysis was restricted to non-obese participants (body mass index (BMI) <26 kg/m^2) aged 20 years and older who performed spirometric tests that met acceptability and reliability criteria of the American Thoracic Society (ATS).[13] To adjust for height, age, sex, and race, we used published prediction equations for FEV_1 and forced vital capacity (FVC) derived from the NHANES population.[14] We divided the cohort into quintile groups based on percentage predicted FEV_1; quintile 1 was defined as FEV_1 ⩽85.2% predicted (mean 70.8%); quintile 2, 85.3–94.3% (mean 90.1%); quintile 3, 94.4–101.4% (mean 98.0%); quintile 4, 101.5–110.0% (mean 105.6%); and quintile 5, ⩾110.1% predicted (mean 118.6%).

Laboratory measurements
Using a radioimmunoassay of polyclonal antibodies raised in rabbits against highly purified recombinant human leptin, leptin concentrations used in this analysis were measured from serum samples of 6415 participants in NHANES 3 who

Table 1 Baseline characteristics of study participants stratified according to severity of FEV$_1$ impairment

	Severity of impairment					
	Quintile 1 (n=561)	Quintile 2 (n=562)	Quintile 3 (n=561)	Quintile 4 (n=562)	Quintile 5 (n=562)	p for trend
FEV$_1$ (% predicted)	70.8 (14.9)	90.1 (2.6)	98.0 (2.1)	100.5 (2.4)	118.5 (8.3)	0.001
FEV$_1$ (l)	2.15 (0.82)	2.95 (0.70)	3.23 (0.77)	3.46 (0.82)	3.53 (1.01)	0.001
FEV$_1$/FVC (%)	69.0 (14.3)	78.5 (8.3)	80.7 (7.8)	82.3 (7.4)	82.3 (7.0)	0.001
Age (years)	53.1 (19.8)	42.8 (18.0)	40.2 (17.9)	39.7 (17.6)	46.3 (21.2)	0.001
BMI (kg/m^2)	22.5 (2.4)	22.6 (2.2)	22.6 (2.2)	22.9 (2.2)	22.9 (2.0)	0.001
Weight (kg)	63.4 (10.6)	64.2 (10.0)	63.9 (9.9)	64.1 (10.0)	63.1 (9.7)	0.685
Males	297 (52.9%)	278 (49.5%)	259 (46.2%)	249 (44.3%)	252 (44.8%)	0.001
Whites	433 (77.2%)	422 (75.1%)	423 (75.4%)	426 (75.8%)	381 (67.8%)	0.002
Current smokers	227 (40.4%)	187 (33.3%)	151 (26.9%)	130 (23.1%)	136 (24.2%)	0.001
Never smokers	213 (38.0%)	275 (48.9%)	301 (53.7%)	324 (57.7%)	321 (57.2%)	0.001
Serum albumin (g/l)	40.9 (3.4)	41.6 (3.5)	41.6 (3.6)	41.9 (3.6)	41.6 (3.5)	0.001
Serum folate (nmol/l)	15.4 (14.2)	14.8 (14.7)	15.6 (15.6)	14.1 (12.3)	15.8 (12.8)	0.952
Total serum cholesterol (mmol/l)	5.3 (1.1)	5.1 (1.1)	5.0 (1.1)	5.0 (1.0)	5.2 (1.1)	0.154
CRP (mg/l)*	3.07	2.70	2.56	2.53	2.52	0.001
Leucocytes ($\times10^3$/µl)	7.07 (2.41)	6.64 (2.09)	6.35 (1.96)	6.34 (1.86)	6.26 (1.97)	0.001
Fibrinogen (g/l)	3.23 (0.88)	3.08 (0.86)	3.03 (0.92)	2.88 (0.82)	2.94 (0.74)	0.001
Triceps skinfold (mm)	13.8 (6.6)	14.7 (6.9)	14.8 (6.6)	14.3 (6.6)	13.2 (6.1)	0.068

Continuous variables are shown as mean (SD) and dichotomous variables are shown as number (% of column totals) unless otherwise indicated.
*Geometric mean.
BMI=body mass index; FEV$_1$=forced expiratory volume in 1 second; FVC=forced vital capacity; CRP=C reactive protein.

provided a morning blood sample after an overnight fast.[15] 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 added to serum was 99–104% over the linear range of the assay.[15] Other measurements (C reactive protein (CRP), fibrinogen, serum albumin, serum folate, and total cholesterol levels) were obtained using standard laboratory techniques which have been previously described.[15]

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/m^2; it was divided into four quartiles (quartile 1, <21.20 kg/m^2; quartile 2, 21.20–22.94 kg/m^2; quartile 3, 22.95–24.49 kg/m^2; and quartile 4, 24.50–25.90 kg/m^2).

Statistical analyses

The baseline characteristics of the study participants across the FEV$_1$ quintile groups were compared using a χ^2 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 FEV$_1$ 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. To test the robustness of our findings in different conditions we divided the serum leptin values into quartiles (<2.9, 2.9–5.2, 5.3–9.6, and >9.6 fg/l) and defined "raised" values as those within in 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 FEV$_1$ 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 2 Log transformed serum leptin values stratified according to severity of lung impairment

	Severity of FEV$_1$ impairment					
	Quintile 1 (n=561)	Quintile 2 (n=562)	Quintile 3 (n=561)	Quintile 4 (n=562)	Quintile 5 (n=562)	p for trend
Log transformed leptin values (fg/l)	1.69 (0.78)	1.67 (0.81)	1.68 (0.78)	1.65 (0.80)	1.60 (0.79)	0.059
Unadjusted partial regression coefficient	0.093 (0.047)	0.069 (0.047)	0.085 (0.047)	0.0565 (0.047)	0.0 (reference)	0.059
Partial regression coefficient adjusted for sex	0.182 (0.034)	0.119 (0.034)	0.099 (0.034)	0.050 (0.034)	0.0 (reference)	0.001
Partial regression coefficient adjusted for sex and BMI	0.250 (0.029)	0.174 (0.029)	0.141 (0.029)	0.056 (0.029)	0.0 (reference)	0.001
Fully adjusted partial regression coefficient†	0.236 (0.029)	0.190 (0.029)	0.160 (0.029)	0.077 (0.029)	0.0 (reference)	0.001

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).
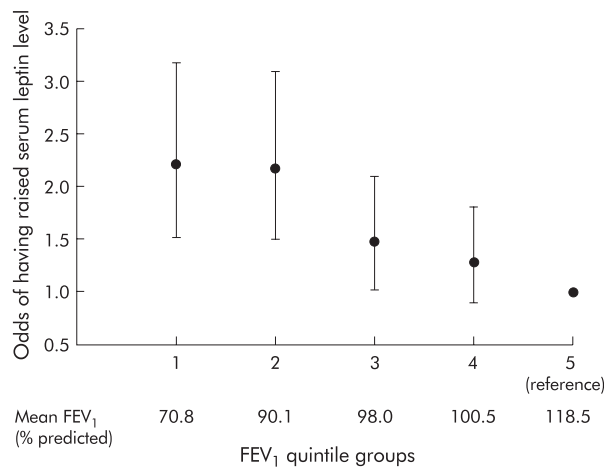BMI=body mass index.

Figure 1 The odds of having a raised serum leptin concentration.

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 (2.2) kg/m^2, respectively. The mean FEV_1, FVC, FEV_1 (% predicted), and FVC (% predicted) were 3.06 (0.97) l, 3.89 (1.10) 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 FEV_1 gradient.

The narrow range of BMI of our cohort allowed us to look for a relationship between FEV_1 and serum leptin levels. There was a strong inverse relationship between FEV_1 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 FEV_1 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 1 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, FEV_1 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.[16] 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.[16] Interestingly, factors such as TNF-α also promote the expression and release of leptin from adipose tissue,[17] indicating a positive feedback mechanism. Since leptin is a potent inflammatory promoter[7] it may exacerbate existing lung and systemic inflammation, contributing to the poor clinical outcomes of patients with various lung diseases.

Table 3 Adjusted relationship of various factors to log transformed serum leptin values

	β coefficient (SE)*	p for trend
FEV_1		
Quintile 1	0.24 (0.03)	0.001
Quintile 2	0.19 (0.03)	
Quintile 3	0.16 (0.03)	
Quintile 4	0.08 (0.03)	
Quintile 5	0.0 (reference)	
Age (years)		
20–29	0.0 (reference)	0.001
30–39	0.01 (0.03)	
40–49	0.13 (0.03)	
50–59	0.11 (0.03)	
60–69	0.22 (0.03)	
70+	0.18 (0.03)	
Body mass index (kg/m^2)		
<21.2	0.0 (reference)	0.001
21.2–22.9	0.35 (0.03)	
23.0–24.4	0.56 (0.03)	
24.5–25.9	0.76 (0.03)	
Sex		
Female	0.0 (reference)	0.001
Male	−1.18 (0.02)	
Race		
White	0.0 (reference)	0.776
Non-white	0.01 (0.02)	
Smoking status		
Ex-smokers	0.0 (reference)	
Current smokers	−0.13 (0.03)	0.001
Never smokers	−0.03 (0.02)	0.302
CRP (mg/l)		
≤2.1	0.0 (reference)	0.001
>2.1	0.09 (0.02)	
Fibrinogen (g/l)†	0.06 (0.02)	0.001
Leucocytes (×10^3/µl)†	0.02 (0.00)	0.001

FEV_1=forced expiratory volume in 1 second; CRP=C reactive protein. All values have been adjusted for age, sex, smoking status, race, body mass index, and lung function (see methods).
*A positive β coefficient denotes an increase while a negative number denotes a decrease in log transformed serum leptin values (in fg/l) compared with reference parameters.
†Change in serum leptin concentration (on a logarithmic scale) for every 1 unit increase.

Leptin is also an important risk factor for coronary events.[18] It has a pressor effect on blood vessels through increased activation of the sympathetic nervous system which is thought to mediate obesity related hypertension.[19] Because excess adrenergic activity has also been noted in advanced lung disease,[20] our findings raise the possibility 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[8] and of osteoporosis through its action on the central hypothalamic pathways.[21] The risks of cardiovascular morbidity and mortality are markedly increased in the presence of low FEV_1.[5] 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.[7][10][11] In one study serum leptin levels were found to be lower in those with advanced lung disease than in healthy controls,[10] a finding that was difficult to understand since, in the same study, TNF-α levels (a positive feedback mediator for leptin[7]) 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[11] 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 FEV_1 impairment 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 FEV_1 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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