Circulatory and Adipose Tissue Leptin and Adiponectin in Relationship to Resting Energy Expenditure in Patients With Chronic Obstructive Pulmonary Disease

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Summary

Increases in resting energy expenditure (REE) likely contribute to weight loss in various chronic diseases. In chronic obstructive pulmonary disease (COPD), relationships between the ventilatory impairment and increased REE, and between disturbances in adipokines and weight loss were previously described. Therefore, we investigated serum levels and adipose tissue expression of leptin and adiponectin, and their relationships to REE in patients with COPD. In 44 patients with stable COPD (38 male; age 62.3±7.2 years), REE was assessed using indirect calorimetry. Subcutaneous adipose tissue samples were analyzed using real-time PCR. From underweight [n=9; body mass index (BMI) <20.0 kg.m^{-2}], to normal weight-overweight (n=24, BMI=20.0-29.9 kg.m^{-2}) and obese patients (n=11; BMI≥30 kg.m^{-2}), REE adjusted for body weight decreased (32.9±6.1 vs. 26.2±5.8 vs. 23.9±6.6 kcal.kg^{-1}.24 h^{-1}, p=0.006), serum levels and adipose tissue expression of leptin increased (p<0.001 for both), and serum and adipose tissue adiponectin decreased (p<0.001; p=0.004, respectively). REE was inversely related to serum and adipose tissue leptin (R=−0.547, p<0.001; R=−0.458, p=0.002), and directly to serum adiponectin (R=0.316, p=0.039). Underweight patients had increased REE compared to normal weight-overweight patients, in association with reductions in serum and adipose tissue leptin, and increased serum adiponectin, suggesting a role of adipokines in energy imbalance in COPD-related cachexia.

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Introduction

Mechanisms responsible for unintentional weight loss in patients with chronic obstructive pulmonary disease (COPD) are poorly understood. Several pathological processes potentially linked to COPD-related wasting were proposed such as energy imbalance resulting from increased energy expenditure and nutritional insufficiency, systemic inflammation coupled with oxidative stress, disuse muscular atrophy, anabolic hormone insufficiency, sympathetic activation, and tissue hypoxia (Wagner 2008, Raguso and Luthy 2011). Nevertheless, despite reduced quality of life and shortened survival time in underweight patients with COPD (Landbo *et al.* 1999, Vestbo *et al.* 2006), studies directly assessing pathological mechanisms potentially involved in COPD-related weight loss are scarce.

Similarly to cancer cachexia (Fouladiun *et al.*

2005), significant loss of the both, fat-free mass and body fat occurs in underweight patients with advanced COPD (Engelen *et al.* 1999, Liu *et al.* 2009). Animal studies in rodents revealed that fat tissue wasting in cachexia is associated with morphological heterogeneity and shrinking of adipocytes accompanied by increased fibrosis in white adipose tissue (WAT) (Bing *et al.* 2006). In addition, extensive delipidation in adipocytes was also observed under cachectic conditions (Murphy *et al.* 2010).

In the last decade the endocrine and secretory function of WAT has been increasingly recognized, and the role of numerous fat cell-derived proteins in the control of various physiological functions has been uncovered. Importantly, adipokines such as leptin are involved also in the control of energy balance (Trayhurn and Bing 2006). Reductions of circulatory leptin levels were reported not only in cancer cachexia (Smiechowska *et al.* 2010) but also in emphysematous or malnourished patients with COPD (Schols *et al.* 1999, Yang *et al.* 2006). In contrast, elevated circulatory adiponectin levels were associated with muscle wasting in cancer cachexia (Wolf *et al.* 2006). Nevertheless, although leptin appeared to increase energy expenditure in mice (Hamann and Matthaei 1996), the effects of leptin on resting energy expenditure (REE) in humans remain poorly understood (Deemer *et al.* 2010). While several studies demonstrated associations between circulatory leptin and REE (Jørgensen *et al.* 1998, Magni *et al.* 2005), other investigations have found no such relationships (Neuhäuser-Berthold *et al.* 2000, Usui *et al.* 2007, Deemer *et al.* 2010).

REE is the major component of total daily energy expenditure in individuals with limited physical activities. Previously, increases in REE were observed in diseased states associated with cachexia other than COPD (Fredrix *et al.* 1991). In addition, elevated REE was observed in patients with COPD (Schols *et al.* 1991a, Creutzberg *et al.* 1998, Sergi *et al.* 2006), with the highest values of REE among weight-losing patients (Schols *et al.* 1991a, Schols *et al.* 1991b).

In patients with COPD, previous findings demonstrated relationships between the severity of ventilatory impairment and increased REE on one hand (Donahoe *et al.* 1989), and between disturbances in circulatory adipokine levels and weight loss on the other (Tomoda *et al.* 2007, Yang *et al.* 2006). Therefore, a question arose regarding the role of adipose tissue and adipokines in REE in such patients. In the present study, we investigated serum levels and adipose tissue expression of leptin and adiponectin, and their relationships to REE in patients with COPD.

Patients and Methods

Subjects

Patients with diagnosis of COPD according to the Global Strategy for Chronic Obstructive Lung Disease (GOLD) recommendations (NHLBI/WHO 2010), free from exacerbation for ≥ 8 weeks, were recruited from two out-patient clinics affiliated with the university hospital setting. Exclusion criteria were respiratory disorders other than COPD, known metabolic, endocrine, autoimmune, hepatic or renal disorders, malignancy, heart failure, blood coagulation disorders or therapy with warfarin, use of systemic corticosteroids and long-term home oxygen therapy. Dyspnoea severity was evaluated using the Modified Medical Research Council (MMRC) scale. Exercise capacity was assessed using 6-minute walking distance (6MWD). The distance travelled within 6 minutes was recorded without use of supplemental oxygen.

Information regarding COPD exacerbations in the preceding year and medication use was retrieved from patients' charts of the referring physicians. The study was a part of an ongoing study on the metabolic consequences of COPD (MOPD) and had approval of the Ethics Committee of the L. Pasteur University Hospital, Kosice, Slovakia. All subjects gave written consent to the study.

Pulmonary function tests

Pulmonary function tests, including spirometry and single-breath diffusing capacity for carbon monoxide (DL_{CO}), were assessed with the use of bodyplethysmography (Ganshorn, Germany) in accordance with European Respiratory Society standards (Miller *et al.* 2005). Tests were performed with patients in a sitting position by the same technician in order to ensure consistency of the technique. Three technically acceptable measurements were performed in each patient, and the highest value was included in the analyses. COPD diagnosis and severity was evaluated on the basis of the GOLD recommendations (NHLBI/WHO 2010).

Body composition

Body composition was assessed by Dual Energy X-Ray Absorptiometry (DEXA) with fan-beam technology using a total body scanner (Lunar Prodigy,

GE Healthcare, United Kingdom). Calibration was performed routinely every morning using the standard provided by the manufacturer. Coefficient of variation during measurement of standard phantom was less than 1 %. Body weight and fat free mass (FFM) were adjusted for height squared to calculate body mass index (BMI) and fat free mass index (FFMI). Patients with BMI<20.0 kg.m^{-2} were considered underweight (Landbo *et al.* 1999, Tomoda *et al.* 2007). Patients with COPD and BMI≥30.0 kg.m^{-2} were diagnosed as obese (Landbo *et al.* 1999, Vestbo *et al.* 2006).

Biochemical analyses

In all patients, peripheral venous blood samples from the antecubital vein were collected between 7.00 and 8.00 a.m. after 10 hour fast. Routine biochemical and hematological assessments were performed at the day of collection. High-sensitivity serum C-reactive protein (hsCRP) levels were assessed by immunoturbidimetric method (Randox, United Kingdom). The analytical sensitivity of this CRP assay is of 0.1 mg.l^{-1}. Serum tumor-necrosis factor (TNF)-α and interleukin (IL)-6 levels were measured using commercially available enzyme-linked immunosorbent assay kits (Beckmann-Coulter Immunotech, Germany). At the time of venous blood samples collection, arterial blood sample was obtained by puncture of radial artery to determine arterial carbon dioxide tension (PaCO$_2$) and arterial oxygen tension (PaO$_2$). Serum leptin and adiponectin were determined by the enzyme-linked immunosorbent assays (DRG, Germany).

Measurements of resting energy expenditure

Resting energy expenditure (REE) was measured by open-circuit indirect calorimetry using a ventilated hood system (ZAN, Germany). The system was calibrated daily. Prior to each metabolic test, the flowmeter was calibrated using a 3 liter calibration syringe (Pulmonary Data Services Inc., USA) and the gas analyzers were calibrated using a two-point calibration method with certified gases (15.9 % O$_2$, 5.0 % CO$_2$, Linde Gas, Slovakia). Patients were examined after at least 10 hours fast, and refraining from smoking, alcohol and caffeine consumption, and vigorous physical activity for 24 hours prior to the test. Patients were placed in a comfortable reclined position, allowed to rest at least 30 min prior to measurement. The measurements were recorded within a 30 min period between 8.00 and 9.00 a.m., and REE was calculated from oxygen consumption

and carbon dioxide production using the abbreviated Weir equation (Weir 1949). Since the absolute values of REE depend on body size, we calculated the ratio of REE to body weight (REE/BW) as previously described (Schols *et al.* 1991b, Sergi *et al.* 2006).

Leptin and adiponectin expression in adipose tissue

Abdominal subcutaneous adipose tissue was taken by aspiration with a bioptic needle (Medin, Czech Republic) under local intracutaneous anesthesia with 1 % mesocain after an overnight fast between 9.00 and 10.00 a.m. The samples were quickly washed in saline to eliminate blood and other connective tissue, immediately frozen in liquid nitrogen and stored at −80 °C until analysis. Total RNA was isolated using the RNeasy® Lipid Tissue mini kit (Qiagen, Germany), DNAse treatment was included. RNA quantity, purity and integrity were determined with the microfluidic chips Experion^{TM} RNA analysis kit (BioRad, USA) as well as with nanophotometer^{TM} (IMPLEN, Germany). Reverse transcription was performed with aid of High Capacity RNA to cDNA kit (Applied Biosystems, USA). Gene expression was measured in duplicates with aid of the real-time PCR (RotorGene 2000 real-time cycler, Corbett Research, Australia) using the TaqMan®Gene Expression Assays for *leptin* **(**Hs00174877_m1) and *adiponectin* (Hs00605917_m1, Applied Biosystems, USA). Comparative quantification method (ΔΔCt) was used to calculate the relative gene expression (Klein 2002).

Statistical analyses

Statistical analyses were performed using SPSS software version 14.0 (SPSS Inc., USA). The Kolmogorov-Smirnov test of normality was applied. Differences between groups in normally distributed variables were tested by one-way analysis of variance (ANOVA) with post hoc testing by all pair-wise multiple comparison procedures using the Tukey test. In non-normally distributed variables, the differences between groups were analysed by ANOVA on ranks using Kruskal-Wallis method and Dunn's method was used for post hoc multiple comparisons. Chi-square test was used to compare the proportion of categorical variables between groups.

Because the distributions of serum hsCRP, TNF-α and IL-6 levels, and of serum leptin and adiponectin concentrations were all skewed, we used the log transformed values of these variables in regression

Table 1. Patient characteristics.

Variable	Entire cohort	Group			p value
		Underweight	Normal weight-overweight	Obese	
Patients, no (%)	44	9 (20.5)	24 (54.5)	11 (25)	
Age, years	62.3 ± 7.2	61.4 ± 8.2	63.4 ± 6.4	60.2 ± 8.0	0.527
Males, no (%)	38 (86.3)	9 (100)	20 (83.3)	9 (81.8)	0.406
Current smokers/ exsmokers, no (%)	19 (43.2)	5 (55.6)	10 (41.7)	4 (36.4)	0.673
Packyears, years	34.0 ± 25.1	40.0 ± 15.0	38.1 ± 29.4	19.2 ± 15.4	0.097
MMRC dyspnea scale	1.62 ± 0.82	2.00 ± 0.71	1.46 ± 0.93	1.64 ± 0.50	0.312
6MWD, m	359.5 ± 90.5	287.7 ± 84.7*	383.9 ± 80.9	365.2 ± 91.3	0.020
BMI, kg.m^{-2}	26.5 ± 7.1	18.2 ± 0.8*†	25.2 ± 2.9	36.0 ± 5.6*	<0.001
FFM, kg	51.8 ± 7.1	46.5 ± 3.4†	51.0 ± 6.3	57.8 ± 7.0*	<0.001
FFMI, kg.m^{-2}	18.3 ± 2.5	16.1 ± 0.8*†	17.8 ± 1.5	21.2 ± 2.6*	<0.001
Fat percentage, %	27.6 ± 12.9	10.6 ± 6.7*†	27.9 ± 8.6	40.6 ± 6.7*	<0.001
Fat mass, kg	22.9 ± 13.9	6.3 ± 4.2*†	21.1 ± 7.5	40.0 ± 10.1*	<0.001

* p<0.05 versus normal weight-overweight group. † p<0.05 versus obese group. Values are given as the mean ± SD. MMRC – The Modified Medical Research Council Dyspnea Scale; 6MWD – 6 minutes walking distance; BMI – body mass index; FFM – fat free mass; FFMI – fat free mass index.

analyses. Least-squares linear regression analysis was used to assess the unadjusted relationships between the REE and serum parameters, and between REE and adipose tissue expression. Pearson product-moment correlation coefficient (R) is reported to show the degree of linear relationship between variables.

In the multivariate analyses, multiple linear regression models were used with REE per kg of body weight as the dependent variable, and age, gender, forced expiratory volume in 1 second (FEV$_1$) to forced vital capacity (FVC) ratio, fat mass, and either log transformed serum leptin, leptin adipose tissue expression, or log transformed serum adiponectin levels as independent variables. Coefficient of determination (R^2) is reported to indicate the model fit, and percentage of variance of the dependent variables explained by the predictors included in the model. Because of an existing co-linearity between leptin adipose tissue expression and fat mass, we calculated a multiple linear regression model without, and with fat mass included as an independent variable.

A *p* value of <0.05 was considered statistically significant. Continuous variables with normal distribution are shown as means ± SD, non-normally distributed variables as median (interquartile range).

Results

Patients

We recruited 44 patients (38 men and 6 women) with stable COPD. They were generally late middle-aged (mean age 62.3±7.2 years) with a mean 34.0±25.1 pack-years history of smoking.

Patients were divided into three groups according to BMI: the first was formed by nine underweight patients (BMI<20.0 kg.m^{-2}), the second by 24 normal weight-overweight (BMI 20.0-29.9 kg.m^{-2}), and the third by 11 obese patients (BMI≥30 kg.m^{-2}).

Table 1 displays demographic data and body composition parameters in the three groups. No differences were observed between the groups in the mean age, proportion of females, smoking history or MMRC dyspnea score. Underweight patients performed significantly lower on the 6 minutes-walking distance test than normal weight-overweight individuals (p<0.05).

From underweight to normal weight-overweight and obese group, FFM, FFMI, fat mass, and fat percentage increased (p for trend <0.001 for all) (Table 1). Underweight patients had significantly lower FFMI, fat mass and fat percentage compared to normal weight-overweight individuals (p<0.05 for all).

Table 2. Pulmonary functions and arterial blood gases.

Variable	Entire cohort	Group			p value
		Underweight	Normal weight-overweight	Obese	
FEV_1, l	1.64 ± 0.75	1.13 ± 0.85	1.78 ± 0.71	1.74 ± 0.62	0.069
FEV_1, % pred	54.0 ± 23.3	$33.8 \pm 19.8*^†$	59.4 ± 22.7	60.5 ± 19.0	0.020
FVC, l	3.1 ± 0.8	2.8 ± 1.1	3.3 ± 0.6	2.9 ± 0.8	0.189
FVC, % pred	83.1 ± 16.9	$68.1 \pm 19.6*$	89.2 ± 12.1	82.0 ± 17.3	0.004
FEV_1/FVC ratio	50.9 ± 16.9	$38.7 \pm 15.2^†$	51.9 ± 17.0	58.6 ± 13.2	0.025
RV, l	4.12 ± 1.39	$5.26 \pm 1.44*^†$	4.01 ± 1.29	3.42 ± 1.04	0.008
RV, % pred	176.3 ± 53.2	$221.0 \pm 59.4*^†$	169.8 ± 47.2	154.1 ± 42.3	0.010
TLC, l	7.28 ± 1.52	$8.14 \pm 1.06^†$	7.34 ± 1.59	6.45 ± 1.32	0.040
TLC, % pred	113.5 ± 17.5	121.2 ± 18.2	114.5 ± 17.8	105.1 ± 13.8	0.110
RV/TLC ratio	55.6 ± 10.5	$64.3 \pm 13.7*^†$	53.7 ± 7.9	52.5 ± 9.5	0.015
DL_{CO}, % pred	72.3 ± 30.9	$34.2 \pm 24.3*^†$	76.3 ± 28.7	92.0 ± 7.6	0.002
PaO_2, kPa	9.17 ± 1.82	$7.83 \pm 2.60*$	9.67 ± 1.57	9.19 ± 1.00	0.032
$PaCO_2$, kPa	5.08 ± 0.88	5.31 ± 1.06	4.94 ± 0.77	5.18 ± 0.96	0.687

* $p<0.05$ versus normal weight-overweight group. † $p<0.05$ versus obese group. Values are given as the mean ± SD. % pred – percentage of predicted value; FEV_1 – forced expiratory volume in 1 second; FVC – forced vital capacity; RV – residual volume; TLC – total lung capacity; DL_{CO} – diffuse capacity for carbon monoxide; PaO_2 – partial pressure of oxygen; $PaCO_2$ – partial pressure of carbon dioxide.

With increases in BMI, FEV_1, FVC, FEV_1/FVC ratio, lung diffusion capacity and PaO_2 significantly increased, whereas residual volume (RV) and RV/total lung capacity (TLC) ratio significantly decreased (Table 2). Underweight patients had significantly more profound obstructive ventilatory impairment (reflected by lower FEV_1), pulmonary hyperinflation (reflected by increases in RV and RV/TLC ratio), and lower lung diffusion capacity compared to normal weight-overweight individuals ($p<0.05$ for all comparisons). In addition, 6MWD correlated directly with FEV_1 and FVC (R=0.484, $p<0.001$; R=0.450, $p<0.002$, respectively), and inversely with RV/TLC (R=−0.481, $p<0.001$).

Resting energy expenditure

From underweight to normal weight-overweight and obese patients, REE/BW significantly decreased (from 32.9±6.1 to 26.2±5.8 to 23.9±6.6 kcal.kg^{-1}.24 h^{-1}, p=0.006) (Fig. 1). In post-hoc analyses, underweight patients had significantly higher REE/BW compared to normal weight-overweight individuals ($p<0.05$). REE/BW correlated inversely with BMI, total fat percentage and fat mass (R=−0.469, p=0.001; R=−0.484, p=0.002; R=−0.538, $p<0.001$, respectively). In addition, REE/BW correlated inversely with FEV_1 and FEV_1/ FVC ratio (R=−0.365, p=0.015; R=−0.317, p=0.036, respectively). Nevertheless,
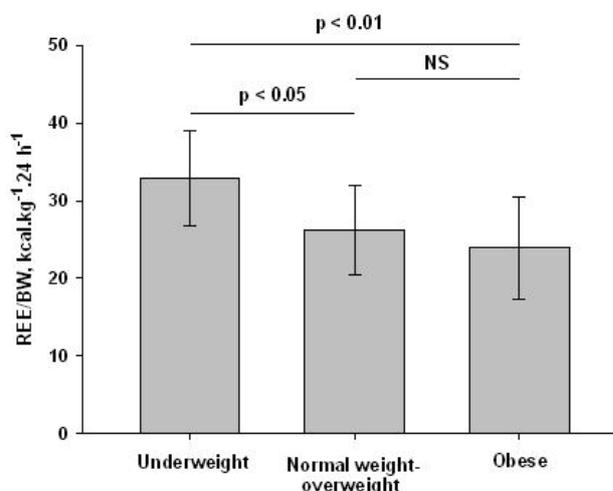

Fig. 1. Comparison of resting energy expenditure per kg of body weight (REE/BW) in patients with COPD (ANOVA, *P* for trend 0.006).

no relationships between REE/BW and 6MWD was observed (R=−0.0106, p=0.946).

Serum markers of inflammation and serum adipokine levels

No differences were observed between the three groups in circulatory leukocytes or neutrophil counts, nor in serum hsCRP, IL-6 and TNF-α levels (Table 3). From

Table 3. Serum parameters of inflammation and serum adipokines levels.

Variable	Entire cohort	Group			p value
		Underweight	Normal weight-overweight	Obese	
Leukocytes, x10^9. l^{-1}	7.00 (5.6-8.2)	6.1 (5.5-8.0)	7.1 (6.3-8.2)	7.0 (5.7-8.8)	0.986
Neutrophils, x10^9. l^{-1}	4.1 (3.3-4.6)	3.3 (3.2-4.9)	4.2 (3.5-4.7)	4.1 (3.3-4.6)	0.705
hsCRP, mg.ml^{-1}	3.10 (1.03-7.75)	3.45 (0.76-7.35)	2.00 (1.05-9.75)	3.60 (1.18-4.60)	0.920
IL-6, pg.ml^{-1}	3.70 (2.90-5.50)	4.30 (3.15-5.15)	3.50 (2.10-5.43)	4.40 (3.00-8.58)	0.385
TNF-α, pg.ml^{-1}	13.40 (10.63-17.50)	9.75 (8.60-14.05)	13.35 (11.00-19.40)	15.20 (11.33-17.28)	0.127
Leptin, ng.ml^{-1}	6.20 (2.05-19.50)	0.60 (0.00-1.60)*[†]	5.45 (2.25-11.65)	22.60 (13.58-43.38)*	<0.001
Adiponectin, µg.ml^{-1}	13.9 (8.9-20.5)	24.8 (18.0-30.8)*[†]	14.0 (9.2-16.8)	9.0 (5.9-11.0)	<0.001

* p<0.05 versus normal weight-overweight group. † p<0.05 versus obese group. Values are given as median (interquartile range). IL-6 – interleukin 6; TNF-α – tumor necrosis factor-alpha; hsCRP – high sensitivity C-reactive protein.

Table 4. Adipose tissue relative expression of leptin and adiponectin in COPD patients.

mRNA expression (ΔΔCt)	Entire cohort	Group			p value
		Underweight	Normal weight-overweight	Obese	
Leptin	39.9 ± 24.6	8.8 ± 9.4*[†]	44.1 ± 20.9	58.1 ± 15.8	<0.001
Adiponectin	184.2 ± 87.4	201.6 ± 100.7[†]	209.4 ± 79.2	107.9 ± 46.2*	0.004

* p<0.05 versus normal weight-overweight group. † p<0.05 versus obese group. Values are given as the mean ± SD.

underweight to normal weight-overweight and obese patients, serum leptin levels significantly increased (p<0.001), whereas adiponectin levels decreased (p<0.001). Underweight patients had significantly lower serum leptin and higher serum adiponectin levels compared to normal weight-overweight individuals (p<0.05 for both). Significant relationships were observed between log transformed serum leptin and adiponectin and FEV$_1$ (R=0.481, p=0.001; R=−0.324, p=0.034, respectively). In contrast, no significant relationships were observed between serum adipokines levels, 6MWD and circulatory concentrations of inflammatory markers hsCRP, IL-6 and TNF-α (data not shown).

Adipose tissue expression of leptin and adiponectin

From underweight to normal weight-overweight and obese patients, adipose tissue mRNA expression of leptin significantly increased, whereas adiponectin expression decreased (p<0.001; p=0.004, respectively) (Table 4). Underweight patients had significantly lower adipose tissue leptin expression compared to normal weight-overweight subjects (p<0.05).

Adipose tissue leptin mRNA expression correlated directly with fat percentage and fat mass (R=0.896, p<0.001; R=0.807, p<0.001, respectively). In contrast, adiponectin expression correlated inversely with fat percentage and fat mass (R=−0.392, p=0.016; R=−0.406, p=0.008, respectively). In addition, adipose tissue leptin expression was related directly to FEV$_1$ and FEV$_1$/FVC ratio, and inversely to RV (R=0.440, p=0.003; R=0.450, p=0.002; R=−0.492, p<0.001, respectively). Nevertheless, no relationships were observed between adipose tissue leptin or adiponectin and 6MWD, or between adipose tissue adiponectin and lung functions.
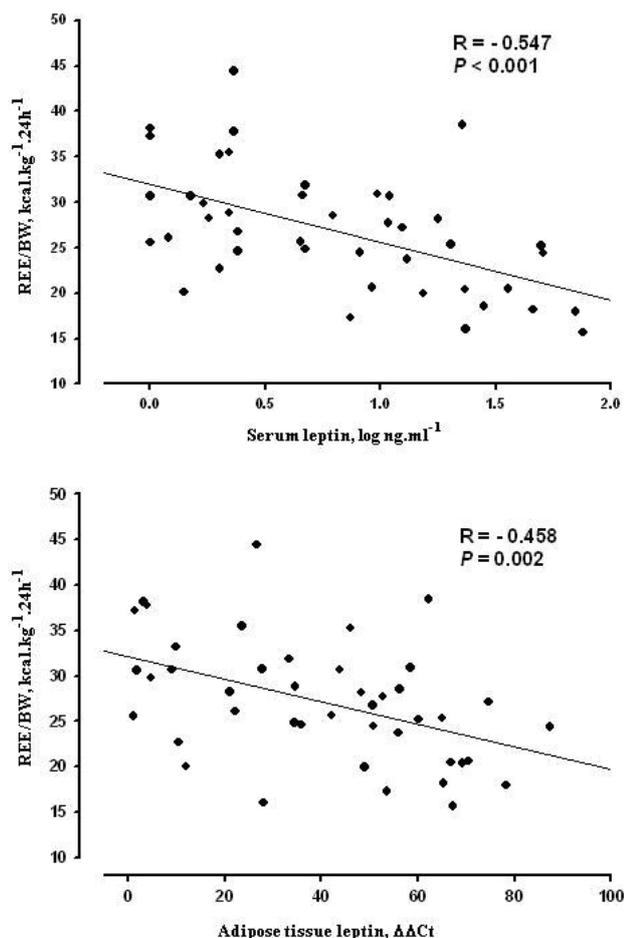
Fig. 2. Relationships between serum and adipose tissue leptin and resting energy expenditure per kg of body weight (REE/BW) in patients with COPD.

Adipokines and resting energy expenditure

In the entire cohort, resting energy expenditure was related inversely to log serum leptin and adipose tissue leptin expression (R=−0.547, p<0.001; R=−0.458, p=0.002) (Fig. 2). In addition, resting energy expenditure was related directly to log serum adiponectin levels, whereas no significant relationship was observed between subcutaneous adipose tissue adiponectin expression and REE/BW (R=0.316, p=0.039; R=0.149, p=0.339, respectively).

In multiple linear regression analysis with REE/BW as the dependent variable and age, gender, FEV_1/FVC ratio, and serum leptin as independent variables, only serum leptin (p=0.016, R^2=0.336) independently predicted REE/BW. In an analogical analysis with serum adiponectin as an independent variable, serum adiponectin and gender were the only independent predictors of REE/BW (p=0.050, p=0.026, respectively; R^2=0.298). Moreover, adipose tissue leptin

independently predicted REE/BW before fat mass was introduced into the multivariate analysis (p=0.043, R^2=0.274). Nevertheless, when fat mass was introduced into this model as the next independent variable, adipose tissue leptin expression did not retain its independent predictive value, and total fat mass represented the only independent predictor of REE/BW (p=0.026, R^2=0.375).

Discussion

The present study provides a novel observation on the role of adipokines in resting metabolic rate in patients with COPD. Our data demonstrate that underweight patients with COPD have increased resting energy expenditure, in association with reductions in their subcutaneous adipose tissue leptin expression and serum leptin levels, and increased serum adiponectin levels. Previous findings in COPD demonstrated relationships between the degree of ventilatory impairment and increased REE (Donahoe *et al.* 1989), and disturbances in circulatory adipokines among those COPD patients who were underweight (Yang *et al.* 2006, Tomoda *et al.* 2007, Liu *et al.* 2009). By concomitant assessment of REE, body composition, circulatory leptin and adiponectin levels and their respective expression within the WAT, our findings are the first to suggest that adipokine levels reflect higher metabolic rate and adipose tissue depletion among patients with COPD and low body weight.

Unexplained weight loss commonly occurs in COPD. In such patients, cachexia is a major clinical problem, which is difficult to reverse, has negative prognostic value, and is associated with serious comorbidities (Wagner 2008). The prevalence of weight loss in COPD increases with disease progression. Significant weight loss experiences up to 15 % of patients with mild-to-moderate disease (Landbo *et al.* 1999), and cachexia occurs in up to 30 % of those with very severe COPD (Vestbo *et al.* 2006). Weight-losing patients with COPD suffer from both, loss of FFM and the loss of FM. This phenomenon has been first described in 1999 by Engelen *et al.* who observed different patterns of chronic tissue wasting among COPD patients with different phenotype. While patients with predominant emphysema suffered from the depletion of the both FFM and FM, patients with predominant chronic bronchitis experienced selective loss of FFM. Likewise, a recent report in underweight patients with COPD and mean BMI of 18.4 kg.m^{-2} demonstrated both, significant depletion of FFM and FM compared to the control group with normal

BMI (Liu *et al.* 2009). Consequently, parallel loss of muscle and fat mass likely occurs at least in some underweight patients with advanced COPD. However, in contrast to increasing number of studies unraveling functional and clinical implications of obesity in COPD (Franssen *et al.* 2008), the data on the consequences of adipose tissue wasting are scarce.

The pathogenesis of weight loss in COPD is multifactorial, including systemic inflammation associated with cytokine-mediated metabolic derangements, tissue hypoxia and oxidative stress (Agustí *et al.* 2004), increased work of breathing resulting in increases in REE (Donahoe *et al.* 1989), disuse muscle atrophy, sympathetic activation, and anabolic hormone insufficiency (Wagner 2008). In such patients, energy imbalance likely results from the both nutritional insufficiency and increased energy expenditure (Schols *et al.* 1991b, Raguso and Luthy 2011). Indeed, several studies demonstrated increases in REE in patients with COPD (Schols *et al.* 1991a, Creutzberg *et al.* 1998, Sergi *et al.* 2006). In agreement with previous reports, results of the present study suggest that increases in resting energy requirements are most profound among malnourished patients (Donahoe *et al.* 1989, Schols *et al.* 1991b). Importantly, Donahoe *et al.* (1989) were the first to describe an association between increased REE, the degree of emphysema and inspiratory muscle weakness, and suggested that increased energy requirements result from augmenting ventilation and increased mechanical work load associated with severe COPD. Our observations demonstrating the most severe ventilatory impairment among underweight patients who were hypermetabolic complement these findings, and extend them further by exploring associations between energy expenditure and adipokine levels.

Leptin, a hormone secreted by adipose tissue, is an integral component of the homeostatic loop of body weight regulation. Leptin acts to control food intake and energy expenditure *via* neuropeptidergic effector molecules within the hypothalamus. While leptin increases metabolic rate in ob/ob mice, these effects are less obvious in wild-type animals (Pelleymounter *et al.* 1995). In addition, human studies on the relationships between serum leptin levels and energy expenditure yielded inconsistent results: some did not reveal any relationship between leptin and REE (Neuhäuser-Berthold *et al.* 2000, Usui *et al.* 2007, Deemer *et al.* 2010), while other reported either a positive (Jørgensen *et al.* 1998) or a negative correlation (Niskanen *et al.* 1997).

Previous studies in COPD patients did not report significant relationships between circulating leptin levels and REE in normal-weight patients with stable COPD (Cohen *et al.* 2003, Shin *et al.* 2007). Some of the inconsistencies observed previously may result from the methodological reasons: since fat mass not only determines serum leptin levels but, besides FFM, also affects REE (Ferraro and Ravussin 1992), analyses of the relationships between leptin and REE require careful adjustments for fat mass (Usui *et al.* 2007). Indeed, in the present study adipose tissue leptin expression was significantly related to resting metabolic rate before adjustments for fat mass, nevertheless, only fat mass predicted REE in the final model. Similar observations suggesting that leptin *per se* does not directly influence REE were recently published in senior women (Neuhäuser-Berthold *et al.* 2000, Usui *et al.* 2007) and men (Neuhäuser-Berthold *et al.* 2000). In the present study we have also observed the lowest adipose tissue expression of leptin in those patients who were underweight. Therefore, reductions of circulating leptin levels in COPD-related cachexia likely result not only from the depletion of adipose tissue *per se* but also from reductions in relative gene expression of leptin within the adipose tissue. Since reductions in leptin levels reflect higher metabolic rate and adipose tissue depletion in underweight patients with COPD, low circulatory leptin concentrations might potentially serve as a biomarker of catabolism in clinical conditions associated with wasting. This hypothesis needs to be addressed by future studies.

Adiponectin is the only adipocytokine with anti-inflammatory and antiobesity effects. Previously, increases in circulatory adiponectin were observed in conditions associated with tissue wasting such as anorexia nervosa (Pannacciulli *et al.* 2003) and cancer cachexia (Wolf *et al.* 2006). In patients with COPD, Tomoda *et al.* (2007) observed remarkable elevations of plasma adiponectin in underweight patients, and speculated that elevations of plasma adiponectin levels may be linked to persistent excess respiratory exercise caused by hyperinflation, and may thus contribute to the development of cachexia. Nevertheless, REE was not measured in that study. Our present observations demonstrating an inverse association between adiponectin and BMI extend previous findings (Tomoda *et al.* 2007, Chan *et al.* 2010) by demonstrating, for the first time, a direct relationship between energy expenditure and serum adiponectin levels among patients with COPD. Recently, parallel increases in REE and adiponectin were reported

after resistance exercise in elderly individuals (Fatouros *et al.* 2009). Nonetheless, the independent predictive value of serum adiponectin on resting energy expenditure in the present study was altered by inclusion of fat mass into the model such that fat mass was the only independent predictor of REE in the final model. Importantly, underweight patients had more severe bronchial obstruction and pulmonary hyperinflation, in association with higher serum adiponectin and REE/BW compared to normal weight-overweight COPD patients. Therefore, it is tempting to hypothesize that chronic excess respiratory exercise due to increased respiratory muscle work load represents one of key pathophysiological mechanisms resulting in concomitant increases in metabolic rate, tissue wasting, and alterations in adipokines in patients with advanced COPD. Studies employing parallel measurements of REE and of respiratory pattern and inspiratory work load using advanced techniques (Dellweg *et al.* 2008) in COPD patients may shed more light on this question.

Previously, several treatment approaches were tested with the aim to reverse weight loss in patients with COPD including active nutritional support, exercise training and optimizing bronchodilator treatment or oxygen supply (Wagner 2008). Nevertheless, improvements achieved by any of these methods were modest in underweight patients (Wagner 2008). The current lack of highly efficient methods to reverse weight loss in COPD coupled with findings of the present study warrant the need to test various weight loss preventive strategies before the initiation of energy expenditure rise associated with disturbances of adipokines, and before the development of manifest cachexia. Addressing these questions is of vital importance for hypermetabolic patients at risk of nutritional insufficiency and subsequent wasting.

There are several limitations to this study. First, only a limited number of patients was studied. However, compared to patients with BMI$\geq$20 kg.m^{-2}, underweight patients with COPD had median values of REE/BW increased by 22 %, serum leptin reduced by 94 %, and serum adiponectin increased by 131 %. Therefore, although our results are robust to gain some understanding on the role of adipokines in COPD-related increases in metabolic rate and weight loss, they should be considered preliminary and hypothesis generating. Second, exploring gender differences in the outcomes of interest was beyond the scope of the present study, and indeed, only six women were recruited. Recently, Breyer

et al. (2011) demonstrated a significant interaction between gender and BMI on serum leptin/fat mass ratio. Therefore, we performed further analyses examining the relationships between adipokines and resting energy expenditure in males only (n=38). Results of these analyses were unequivocally similar to those observed in the entire cohort: serum and adipose tissue leptin were related inversely to REE/BW (R=−0.435, p=0.007; R=−0.372, p=0.022, respectively), while serum adiponectin was related directly to REE/BW (R=0.395, p=0.016). Measurements of adipokines' expression in samples from subcutaneous depots of adipose tissue only represents the third limitation since the expression of leptin and adiponectin may differ between subcutaneous and visceral adipose tissue. In particular, adiponectin is primarily produced by visceral adipose tissue (Motoshima *et al.* 2002). Consequently, circulating adiponectin levels more reliably reflect visceral compared to subcutaneous adipose tissue production of adiponectin. Indeed, while we observed a significant relationship between serum adiponectin and REE, no such relationship was observed when adipose tissue adiponectin expression was introduced into the analysis. Final limitation of the study relates to its cross-sectional nature that does not allow for the determination of time-course relationship between REE, weight loss and adipokines. Nevertheless, concomitant measurements, within one group of patients with COPD, of REE, body composition, and of serum and adipose tissue adipokines are unique and represent strength of the study.

In conclusion, our study highlights the role of adipose tissue and adipokines in energy expenditure in patients with COPD. The observation of alterations in adipokine levels and increased metabolic rate among underweight patients, in association with relationships between leptin and adiponectin and resting metabolic rate in the entire cohort are novel, and suggest that fat metabolism is a factor linked to metabolic rate in patients with COPD. Importantly, the effects of the both, leptin and adiponectin on resting metabolic rate do not appear to be independent of fat mass and, therefore, both hormones likely represent fat-tissue derived mediators of energy expenditure. Further studies are needed to address the pathological mechanisms underlying the observed relationships in more details.

Conflict of Interest
There is no conflict of interest.

Acknowledgements

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