Plasma Leptin; Tumor Necrosis Factor-α and Nutritional Status in Chronic Obstructive Pulmonary Disease Patients

'Tawfik El Adl; **Hossam Eldin Hamza; ***Mohammed Hussin; ****Mostafa Neamat-Allah.

* Lecturer of Internal Medicine, Benha University; ** Lecturer of Chest Medicine, Benha University; *** Lecturer of Chest Medicine, Benha University; **** Lecturer of Medical Biochemistry, Mansoura University.

ABSTRACT

Introduction: Chronic obstructive pulmonary disease (COPD) is a syndrome of chronic wasting, in part associated with a chronic inflammatory response. Leptin is a protein mainly secreted by adipocytes, and the major function of leptin is its role in control of body weight and energy expenditure. It is suggested that increased levels of circulating leptin may contribute to anorexia in pathologic conditions including COPD. Several studies have provided evidence for a link between leptin and proinflammatory cytokines such as tumor necrosis factor-α (TNF-α).

Aim of work: This study aimed to investigate, prospectively, the potential role of circulating plasma leptin and TNF-α levels in the malnutrition of COPD patients, and to observe the changes of serum leptin levels during acute exacerbations. Also to investigate the relationship between leptin and TNF-α in COPD patients; and to determine whether plasma leptin levels are related to body mass index or to TNF-α levels.

Patients & Methods: Sixty COPD patients and ten healthy control subjects participated in this study. Sixty COPD patients were divided into 3 groups: Group I: Patients without malnutrition, during acute exacerbations (n=20); Group II: Patients without malnutrition, during stable disease (n=20); and Group III: Patients with malnutrition during stable disease (n=20). To eliminate the effect of sex differences, all patients and controls were males. Body mass index (BMI), triceps skin-fold thickness (TSF), mid-upper arm circumference (MAC), mid-upper arm muscle circumference (MAMC), serum leptin and TNF-α levels, serum transferrin (TF), serum albumin (Alb), serum prealbumin, total lymphocytes count (TLC), forced expiratory volume in one second (FEV1), maximal inspiration pressure (MIP) and maximal expiration pressure (MEP) were measured in all participants. Leptin levels were measured by ELISA; TNF-α levels were measured by ELISA. The between group difference and correlation of these parameters were analyzed.

Results: Nutritional parameters were significantly lower in Group III than other groups (P<0.05). Serum leptin levels were significantly lower in Group III (COPD, with malnutrition, stable disease) [(12.2±2.5) ng/ml] than in Group II (COPD, without malnutrition, stable disease) [(9.7±5.3) ng/ml] and controls [(12.2±2.5) ng/ml] (P<0.05). However, the difference between Group I (COPD, without malnutrition, acute state), Group II (COPD, without malnutrition, stable disease), and controls was not statistically significant (P>0.05). There was no statistically significant difference in serum TNF-α levels between Group I, Group II, Group III and controls (P>0.05). There was no significant correlation between leptin and TNF-α in any group.

Conclusions: Leptin was not involved in anorexia and weight loss of COPD patients. There was no statistically significant difference in serum leptin levels between COPD patients during stable stage and acute exacerbation, and there was no significant correlation between TNF-α and leptin during the regulation of the energy balance in COPD patients. In addition, circulating leptin works independently of the TNF-α system and does not primarily affect BMI in COPD patients.

Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by the progressive development of airflow limitation that is not fully reversible. The clinical syndrome of COPD encompasses different disease conditions varying from chronic obstructive bronchitis with obstruction of small airways, to emphysema characterized by enlargement of air spaces and destruction of lung parenchyma, loss of lung elasticity, and closure of small airways (Wouters, 2002, and Yang et al., 2006).
Unexplained weight loss commonly occurs in patients with chronic obstructive pulmonary disease (COPD); independent of the severity of airflow obstruction (Takabatake et al., 1999). The frequent occurrence of weight loss and subsequent tissue depletion in COPD patients is associated with an increased morbidity and even mortality. Although the increased work of breathing in COPD could be partly responsible for excessive energy expenditure, this hypothesis is still controversial (Hugli et al., 1995). In either case, it does not by itself explain weight loss, and other mechanisms have been considered (Takabatake et al., 1999).

In addition, several reports have provided evidence that weight loss also negatively affects the prevalence and outcome of acute disease exacerbations of COPD. Weight loss and loss of fat mass are primarily the result of a negative balance between dietary intake and energy expenditure, while muscle wasting is a consequence of an impaired balance between protein synthesis and protein breakdown (Schols et al., 1998, and Yang et al., 2006). Weight loss may involve all tissue compartments, although loss of skeletal muscle may be particularly important because of wasting of respiratory muscles with loss of power and endurance (Nishimura et al., 1995). The increased work of breathing in COPD could be partly responsible for excessive energy expenditure. An elevated energy metabolism not adequately met by an increased spontaneous dietary intake underlies weight loss in COPD. Inflammatory activity probably contributes to catabolic processes (Figen et al., 2005).

Several plasma proteins of hepatic origin, such as albumin, prealbumin, and retinol binding protein, in addition to 24-hour urinary creatinine excretion in individuals with normal renal function (correlates well with total body muscle mass); have been suggested to be good dynamic markers and good biochemical indices of nutritional status. Changes in the plasma concentration of these proteins may reflect changes in nutritional status (Figen et al., 2005).

Leptin is a 16-kilodalton adipocyte-derived hormone that is encoded by the ob gene and circulates in the systemic blood in the free and bound form. Circulating levels of leptin reflect the amount of energy stored in adipose tissue and play an important role in energy balance and fat mass regulation.

Centrally, leptin acts either directly, or by binding to specific receptors (Ob-Rb) in the hypothalamus, to reduce neuropeptide-Y (NPY) and agouti regulated protein (AgRP) activity, to increase pro-opiomelanocortin (POMC) and cocaine- and amphetamine related protein (CART) neuron activity, effectively reducing appetite and feed intake. Administration of leptin results in a reduction of food intake as well as increasing energy expenditure. Deficiencies in leptin signalling or functioning in the hypothalamus are thought to contribute to the development of obesity (Bjorbaek & Kahn, 2004).

In peripheral tissues, leptin generally has a fat metabolising role with limited direct effect on glucose metabolism. However, leptin antagonises insulin action and decreases its production by pancreatic β-cells (Seufert, 2004), it indirectly affects glucose metabolism, for example glucose transport in skeletal muscle via the hypothalamus and central nervous system (Minokoshi et al. 2002).
Evidence suggests that leptin increases lipolysis in adipose tissue and cells, and in skeletal muscle, but it appears less critical to liver function. Leptin receptor signalling in peripheral tissues is not well understood but involves various transcription factors, such as activation of signal transducer and activator of transcription 1 (STAT1) and STAT3 in adipose tissue and of STAT3 and Akt in skeletal muscle. Furthermore, the increase in fatty acid oxidation by leptin in skeletal muscle has been attributed to the activation of the cellular nutrient-sensing AMP-activated protein kinase (AMPK) signalling pathway (Minokoshi et al. 2002, and Steinberg et al. 2003).

Leptin alters neuroendocrine function, and increases thermoregulatory energy expenditure by altering sympathetic and parasympathetic nervous system activities. In other words, improvements in lipid metabolism and glucose homoeostasis, and increased thermogenesis, are considered to be some of the important metabolic effects of leptin (Mantzoros, 1999, and Takabatake et al. 2001).

In other words, after interaction with specific receptors located in the central nervous system and in peripheral tissues, leptin induces a complex response, including control of body weight and energy expenditure (Tartaglia, 1997, and Wouters, 2002). In animal models, the result of this interaction is a decrease in food intake. Administration of recombinant leptin to ob/ob mice, which have a genetic defect in leptin production, reduces food intake, increases energy expenditure, and decreases body weight (Auwerx & Stael, 1998).

Circulating leptin levels are reported to correlate with the body mass index (BMI) in humans. In pathologic conditions such as chronic renal insufficiency, and bacterial endotoxemia, and with exposure to high-dose glucocorticoids, inappropriately increased levels of leptin are thought to induce excessive metabolic effects underlying anorexia and loss of body weight (Merabet et al., 1997, and Takabatake et al., 1999).

Besides its function in energy homeostasis, leptin plays an important role in T cell mediated immunity, angiogenesis, reproduction, and ventilatory control (O'Donnell et al., 1999).

It has been demonstrated that circulating leptin levels show significant ultradian and circadian variation with a distinct nocturnal peak, although the mechanisms regulating leptin production in the adipose tissue and its potential physiologic significance remain unknown (Licinio et al., 1998).

In view of the fact that both anorexia and hypermetabolism play a role in the wasting associated with chronic pulmonary disease, underlying abnormalities in the leptin feedback mechanism might also be involved. In particular, elevated concentrations of circulating leptin or, on the contrary, a hypothalamic insensitivity to a decrease in leptin concentrations might be present (Takabatake et al., 1999).

The observed link between inflammatory cytokines and leptin in experimental animal studies led to the hypothesis that adipose tissue gene expression is regulated by
inflammatory cytokines, which in turn could induce anorexia in acute or chronic inflammation. Several reports have furthermore shown an enhanced production of tumor necrosis factor-α (TNF-α) in patients with COPD suffering from weight loss (Grunfeld et al., 1996).

Wasting of body cell mass is an important and serious systemic manifestation of COPD; as a loss of more than 40% of actively metabolizing tissue is incompatible with life. Several studies have provided clear evidence for involvement of TNFα in the pathogenesis of tissue depletion in patients with COPD. Increased plasma levels of TNFα and soluble TNF receptors were found in patients with COPD, particularly those suffering from weight loss (Creutzberg et al., 2000a).

It has been suggested that tumor necrosis factor-α (TNF-α), a pleiotropic cytokine causing cachexia, plays a part in metabolic changes associated with chronic wasting diseases such as cancer, cystic fibrosis, congestive heart failure (CHF), and COPD (Engelen et al., 2001, and Yang et al., 2006).

It was shown that both circulating levels of TNF-α, and TNF-α production by peripheral blood monocytes were increased in weight-losing COPD patients, suggesting that activation of the TNF-α system was associated with the cachexia observed in COPD patients. Importantly, administration of endotoxin or cytokines including TNF-α or IL-1 produced a prompt and dose-dependent increase in serum leptin levels in both experimental animals and in humans. This suggests that increased levels of circulating leptin may contribute to anorexia and weight loss in pathologic conditions including COPD (Takabatake et al., 2001).

Some studies have shown a direct relationship between TNFα levels and resting metabolic rate, whereas others have reported an association between resting metabolic rate and raised levels of acute phase proteins. In one study hypermetabolic patients with an acute phase response had a significantly lower fat free mass than hypermetabolic patients without the acute phase reaction despite a comparable body mass index, indicating that systemic inflammation may cause hypermetabolism and induce a catabolic response (Nguyen et al., 1999, and Engelen et al., 2000).

It seems that inflammatory mediators such as TNFα is likely to affect skeletal muscle regulation at two phases: (1) by inhibition of the formation of new myofibres and (2) by degeneration of newly formed myotubes and by the inability to repair damaged skeletal muscle (Wouters, 2002).

The association of an abnormal inflammatory response of the lungs to noxious particles or gases with airflow limitation in COPD indicates the critical role of the inflammatory process in the pathogenesis of this disease (Pauwels et al., 2001).

Changes in a number of proinflammatory mediators such as tumour necrosis factor (TNF)α and interleukin (IL)-8 as well as increased levels of acute phase proteins have been reported, even in patients with stable COPD (Sauleda et al., 2000).
Exacerbations of COPD seem to be particularly associated with increased bronchial and systemic inflammation (Roland et al., 2001). In general, the course of an inflammatory process will be determined by the balance between pro- and anti-inflammatory mediators. One study assessed systemic levels of the anti-inflammatory mediators soluble IL-1 receptor type II (sIL-1RII), the decoy receptor of IL-1, and of the soluble TNF receptors 55 and 75 (sTNF-55 and sTNF-R75) which inhibit the biological activity of TNF. It was found that, in patients with stable COPD, sTNF-R55 was significantly increased compared with controls and sTNF-R75 showed a tendency to be increased. No differences were seen in circulating sIL-1RII levels between patients with COPD in a stable condition and controls. However, treatment of exacerbations was shown to be associated with an increase in sIL-1RII levels which could play a role in the clinical improvement of these patients (Dentener et al., 2001).

Several studies have shown a preferential loss of muscle mass in patients with COPD, especially in the lower extremities. There is now evidence that the ATP dependent ubiquitin-proteasome pathway is responsible for most of the increased proteolysis in various types of muscle atrophy. Several adaptations indicating activation of the ubiquitin-proteasome pathway have been found and these appear to be common to many different forms of muscle atrophy (Jagoe & Goldberg, 2001).

Direct effects of TNFα on differentiated skeletal muscle cells were reported by Li et al. The TNF signal was transduced in part by activation of NF-κB and TNFα rapidly stimulates ubiquitin conjugation to muscle proteins (Li et al., 1998).

Langen et al evaluated the effects of the inflammatory cytokines TNFα and IL-1β on myocytes and found that TNF induced NF-κB activation interfered with the expression of muscle proteins in differentiating myoblasts; the activity of muscle creatine kinase and the amount of myosin heavy chain (MyHC) decreased significantly after 72 hours of exposure to TNFα (Langen et al., 2002).

**Patients & Methods**

This study was carried out at General Private Hospital located in Abu Dhabi, UAE, for a period starting from January 2005 till January 2006. Seventy cases were recruited from the out-patient clinic of internal medicine at their routine consultation. All patients provided informed consent after receiving a full explanation of the nature and protocol of the study. The study protocol was approved by the medical ethical committee of the hospital.

Sixty patients with chronic COPD (selected prospectively and consecutively) and ten age-matched healthy control subjects participated in this study.
The sixty selected cases were classified as follows:

* **Group I** (20 cases): Chronic COPD patients, without malnutrition, during acute exacerbation.
* **Group II** (20 cases): Chronic COPD patients, without malnutrition, during stable disease.
* **Group III** (20 cases): Chronic COPD patients, with malnutrition, during stable disease.
* **Group IV** (10 cases): Control group, healthy subjects coming for routine check up.

**Patients Inclusion Criteria**
Sixty cases with chronic COPD stratified into emphysema (32 patients) and chronic bronchitis (28 patients) were enrolled in this study, and all were males. The diagnosis of COPD was based according to the criteria of the American Thoracic Society guidelines (1995), and when they fulfilled the following criteria: (1) chronic airflow obstruction defined as a measured forced expiratory volume in one second (FEV1) less than 70% of the reference value; (2) irreversible chronic obstructive airway disease (confirmed by spirography), i.e., <10% improvement in FEV1, expressed as % of predicted after inhalation of a beta2-agonist and (3) Post-bronchodilator FEV1/FVC ≤ 0.7, confirms the presence of airflow limitation that is not fully reversible. Spirometric classification was evaluated in all patients (Celli et al., 2004). Patients were considered with malnutrition, if their body mass index (BMI) < 20 kg/square meter (Veldee, 1994). Patients were considered in COPD with clinically stable condition, if they: 1-were not suffering from a respiratory tract infection or heart failure; 2-had no exacerbation of respiratory symptoms in the last 3 months; and 3-had not changed their medications 2 months before the study (Annemie et al., 1999).

All patients were receiving inhaled beta-2 agonists, inhaled ipratropium bromide, some were using inhaled corticosteroid treatment (31 patients). Systemic steroid treatment was not used by any patient. The patients were not receiving any nutritional support therapy. To eliminate the effect of sex differences and to increase homogeneity of the study, all patients and controls were males.

**Patients Exclusion Criteria:**
Patients who had conditions known to affect serum leptin or TNF-α levels, such as systemic corticosteroid use, cancer, collagen vascular disease, cardiac failure, renal failure, gastrointestinal disorders or diabetes and other endocrinopathies or recent surgery and smoking; were strictly excluded. Also, patients with abnormal fluid balance as manifested by the presence of oedema or regular use of diuretics were also excluded (Takahatake et al., 1999).

The 10 age-matched healthy volunteer male control subjects were non-smokers, had no history of medical illnesses (endocrinal, renal, hepatic, or other metabolic disorder), had normal physical examination, blood counts, chemistries, and showed no symptoms or signs of infection at the time of study. All participants were informed about the nature of the study and all consented to participate.

The following was done for all patients included in the study (all were performed at the same setting):
• A thorough history taking.
• A thorough clinical examination; some anthropometric indices and some nutritional parameters were assessed and included the following:

  - Body mass index (BMI) = body weight in kg/height in square meter (Kg/m$^2$).
  - Percent ideal body weight (IBW%).
  - Triceps skin-fold thickness (TSF).
  - Mid-upper arm circumference (MAC).
  - Mid-upper arm muscle circumference (MAMC).

Percent of ideal body weight was calculated using the 1995 National Nutritional Assessment by the Japanese Ministry of Health and Welfare. Height (in square meter) was estimated with subjects standing barefoot (determined to the nearest 1.0 cm-Lameris WM 715; Breukelen, Netherlands) and body weight (kg) was measured with light cloths using the same beam scale to the nearest 1.0 kg (SECA, FRG, Germany) (Annemie et al., 1999). Assessment of individuals nutritional status was done by measurement of BMI; some biochemical and anthropometric markers of nutrition (Figen et al., 2005). BMI values of <20 were accepted as malnourished underweight (Veldee, 1994).

• Pulmonary function tests
  FEV1, FVC, maximal inspiration pressure (MIP) and maximal expiration pressure (MEP) were measured with standard spirometric techniques and tests according to national guidelines using (Vmax62 Sensor Medics, USA). The highest value from at least three spirometric maneuvers was used and expressed as a percentage of predicted (Quanjer, 1993). Degree and reversibility of airway obstruction were assessed according to GOLD (2001). Indices of airflow obstruction, FEV1 and FEV1/FVC were measured. FEV1 and FVC were expressed as percentage of predicted values (FEV1% and FVC%) according to the prediction equation of the European Respiratory Society (Quanjer, 1993).

• Laboratory investigations
  Blood samples were drawn from all subjects by venipuncture in the early morning (8:30 AM) and who had been fasting 12 hours since the previous night. Both serum and plasma were separated from blood cells by centrifugation at 1000×g for 5 minutes. All samples were stored at -70°C until analyzed. Blood samples from COPD patients in acute exacerbation were taken before the start of steroid treatment. The following were measured:

  - Plasma leptin levels were detected using double antibody sandwich ELISA assay kits (Diagnostic Systems Lab. DSL Webster, Texas, USA).
  - Serum TNF-α levels were measured by commercially available specific ELISA kits (Diaclone, France) according to the manufacturers' instructions. All analyses and calibration were performed in duplicate.
  - Serum albumin levels were assessed by an Olympus AU 600 autoanalyzer.
  - Serum prealbumin (PA) and,
- Serum transferrin (TF) were measured by immune rate nephelometry (IRN) (Beckman, USA)
- Total lymphocyte count (TLC).

Statistical analysis

All data were recorded on an investigative report form. Data were expressed as mean ± standard deviation (SD). P<0.05 level considered statistically significant. ANOVA test was done for comparison of the differences among groups. All data were transferred to IBM-card using IBM-PC with analysis of data by a statistical program: SPSS (Statistical Package for the Social Sciences) software package, V 9.05 (USA-1998-echo soft corporation). Correlations between variables were evaluated by Pearson’s correlation coefficient.

Results

The anthropometric characteristics of the studied cases (n=70) are represented in table (1).

<table>
<thead>
<tr>
<th></th>
<th>Chronic COPD (n=60)</th>
<th>Control (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Range</td>
</tr>
<tr>
<td>Age (years)</td>
<td>50.2±8.3</td>
<td>32-62</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>21.0±2.3</td>
<td>18.5-27</td>
</tr>
<tr>
<td>IBW( % )</td>
<td>101.1±12.6</td>
<td>80-122</td>
</tr>
<tr>
<td>TSF (mm)</td>
<td>10.5±3.3</td>
<td>5-16.5</td>
</tr>
<tr>
<td>MAC (cm)</td>
<td>26.4±2.2</td>
<td>22-30.5</td>
</tr>
<tr>
<td>MAMC (cm)</td>
<td>23.0±1.8</td>
<td>24.8-21.2</td>
</tr>
</tbody>
</table>


Lung function measurements of both the COPD patients and the healthy controls are shown in Table (2). The levels of FEV1(% pred), FVC(% pred), FEV1/FVC, MIP(% pred) and MEP(% pred) were significantly lower (P<0.05) in COPD patients than in healthy control. The COPD patients had moderate/severe airway limitation. The control subjects had normal FVC and FEV1 on spiromgrams. 24/60 (40%) patients were presented with moderate degree of COPD, while 36/60 (60%) patients were presented with severe degree of COPD.
**Table (2):** The Pulmonary Function Tests of the studied cases (n=70):

<table>
<thead>
<tr>
<th></th>
<th>Chronic COPD (n=60)</th>
<th>Control (n=10)</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁ (% of predicted)</td>
<td>51.2±12.4</td>
<td>91.1±6.3</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>20-72</td>
<td>80-100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FVC (% of predicted)</td>
<td>73.0±15.2</td>
<td>91.9±7.1</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>50-100</td>
<td>80-100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>FEV₁/FVC</td>
<td>54.5±14.5</td>
<td>74.6±4.8</td>
<td>&lt;0.001</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>40-69</td>
<td>69.8-79.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MIP (% of predicted)</td>
<td>52.4±17.8</td>
<td>71.6±6.2</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>25-91</td>
<td>65-80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MEP (% of predicted)</td>
<td>41.8±8.5</td>
<td>42.5±10.6</td>
<td>&lt;0.05</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>25-61</td>
<td>27-55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

FEV₁: forced expiratory volume in one second, FVC: Forced vital capacity, MIP: maximal inspiration pressure, MEP: maximal expiration pressure. HS= highly significant, S= significant, NS= insignificant.

The laboratory investigations of the studied cases are shown in table (3); where the plasma leptin levels were significantly lower (P<0.01) in those patients with chronic COPD when compared to control group. Also, the TNF-α levels are significantly lower (P<0.05) in those patients with chronic COPD when compared to control group.

**Table (3):** The Laboratory tests of the studied cases (n=70):

<table>
<thead>
<tr>
<th></th>
<th>Chronic COPD (n=60)</th>
<th>Control (n=10)</th>
<th>P value</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean±SD</td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>Range</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leptin (ng/ml)</td>
<td>8.6±4.5</td>
<td>12.2±2.5</td>
<td>&lt;0.01</td>
<td>HS</td>
</tr>
<tr>
<td></td>
<td>0.7-17.2</td>
<td>6.2-14.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α (pg/ml)</td>
<td>8.2±2.2</td>
<td>10.2±3.6</td>
<td>&lt;0.05</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>4.6-13</td>
<td>5.8-14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TLC (x10⁹/L)</td>
<td>2.6±0.6</td>
<td>2.3±0.3</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>1.7-3.5</td>
<td>1.8-2.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alb (g/dl)</td>
<td>4.0±0.4</td>
<td>4.1±0.5</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>3-5.2</td>
<td>3.6-5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA (mg/dl)</td>
<td>25.1±6.1</td>
<td>25.6±4.1</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>11.8-33</td>
<td>18.3-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TF (mg/dl)</td>
<td>239.7±40.1</td>
<td>286.0±30.5</td>
<td>&gt;0.05</td>
<td>NS</td>
</tr>
<tr>
<td></td>
<td>170-320</td>
<td>235-315</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

TNF-α: Tumor necrosis factor-α, TLC: Total lymphocyte count, Alb: albumin, PA: Prealbumin, TR: Transferrin HS= highly significant, S= significant, NS= insignificant.

Lung function measurements in the COPD patients and the healthy controls are shown in table (4). There was no statistically significant difference (P>0.05) in the proportion of moderate and severe COPD between Group I (COPD, without malnutrition, acute state), Group II (COPD, without malnutrition, stable disease) and Group III (COPD, with malnutrition, stable disease).
MIP in Group III and Group II was significantly lower than in controls \((P<0.001)\). However, there was no statistically significant difference \((P>0.05)\) in MIP between group Group III and Group II.

**Table (4):** Lung function measurements in the COPD patients and the healthy controls:

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=20) Mean ±SD</th>
<th>Group II (n=20) Mean ±SD</th>
<th>Group III (n=20) Mean ±SD</th>
<th>Control (n=10) Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>FEV(_1) (% of predicted)</td>
<td>53.3±12.4</td>
<td>47.0±10.3</td>
<td>53.2±14.5</td>
<td>91.1±6.3</td>
</tr>
<tr>
<td>FVC (% of predicted)</td>
<td>72.3±18.3</td>
<td>77.3±13.5</td>
<td>69.5±13.8</td>
<td>91.9±7.1</td>
</tr>
<tr>
<td>FEV(_1)/FVC</td>
<td>50.9±13.9</td>
<td>55.5±14.6</td>
<td>45.7±13.6</td>
<td>74.6±4.8</td>
</tr>
<tr>
<td>MIP (% of predicted)</td>
<td>70.8±16.9</td>
<td>42.2±8.1</td>
<td>44.1±10.1</td>
<td>71.6±6.2</td>
</tr>
<tr>
<td>MEP (% of predicted)</td>
<td>46.5±11.4</td>
<td>39.6±7.3</td>
<td>39.3±3.8</td>
<td>42.5±10.6</td>
</tr>
<tr>
<td>Moderate COPD (n)</td>
<td>12</td>
<td>6</td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td>Severe COPD (n)</td>
<td>8</td>
<td>14</td>
<td>14</td>
<td>-</td>
</tr>
</tbody>
</table>

FEV\(_1\): forced expiratory volume in one second, FVC: Forced vital capacity, MIP: maximal inspiration pressure, MEP: maximal expiration pressure.

**Table (5):** The nutritional parameters in the COPD patients, and the healthy controls:

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=20) Mean ±SD</th>
<th>Group II (n=20) Mean ±SD</th>
<th>Group III (n=20) Mean ±SD</th>
<th>Control (n=10) Mean ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m(^2))</td>
<td>23.4±1.5</td>
<td>23.3±1.4</td>
<td>19.4±0.8</td>
<td>23.9±1.7</td>
</tr>
<tr>
<td>IBW(%)</td>
<td>107.3±8.3</td>
<td>109.0±8.5</td>
<td>86.9±5.7</td>
<td>105.3±7.8</td>
</tr>
<tr>
<td>TSF (mm)</td>
<td>11.8±2.6</td>
<td>12.6±2.7</td>
<td>7.2±1.7</td>
<td>12.3±3.2</td>
</tr>
<tr>
<td>MAC (cm)</td>
<td>27.1±1.4</td>
<td>28.0±1.6</td>
<td>24.0±1.5</td>
<td>26.4±2.7</td>
</tr>
<tr>
<td>MAMC (cm)</td>
<td>23.7±1.8</td>
<td>24.5±1.8</td>
<td>21.1±3.9</td>
<td>23.7±1.8</td>
</tr>
<tr>
<td>TLC (x10(^9)/L)</td>
<td>2.8±0.6</td>
<td>2.7±0.5</td>
<td>2.2±0.5</td>
<td>2.3±0.3</td>
</tr>
<tr>
<td>Alb (g/dl)</td>
<td>3.9±0.2</td>
<td>4.3±0.5</td>
<td>3.9±0.4</td>
<td>4.1±0.5</td>
</tr>
<tr>
<td>PA (mg/dl)</td>
<td>23.0±5.6</td>
<td>28.9±3.8</td>
<td>23.4±7.0</td>
<td>25.6±4.1</td>
</tr>
<tr>
<td>TF (mg/dl)</td>
<td>240.9±29.5</td>
<td>267.1±36.1</td>
<td>211.3±35.8</td>
<td>286.0±30.5</td>
</tr>
</tbody>
</table>


The nutritional parameters in the COPD patients and the healthy controls, are shown in table (5). BMI and IBW\% were significantly lower in Group III (COPD, with malnutrition, stable disease) than in Group I (COPD, without malnutrition, acute state). Group II (COPD, without malnutrition, stable disease), and controls \((P<0.001)\). TSF, MAC, MAMC, TF, Alb were significantly lower in Group III than in Group II and controls \((P<0.001)\). TLC, PA were significantly lower in Group III than in Group II \((P<0.01)\).
Table (6): Serum leptin levels were significantly lower in Group III (COPD, with malnutrition, stable disease) \((5.7\pm 2.7)\) ng/ml than in group Group II (COPD, without malnutrition, stable disease) \((9.7\pm 5.3)\) ng/ml and controls \((12.2\pm 2.5)\) ng/ml \((P <0.05)\). However, the difference between Group I (COPD, without malnutrition, acute state), Group II (COPD, without malnutrition, stable disease), and controls was not statistically significant \((P >0.05)\).

There was no statistically significant difference in serum TNF-\(\alpha\) levels between Group I, Group II, Group III and controls \((P >0.05)\), as shown in table (6), Figure 1 and 2.

**Table (6):** Serum leptin, and TNF-\(\alpha\) in the COPD patients and the healthy controls:

<table>
<thead>
<tr>
<th></th>
<th>Group I (n=20)</th>
<th>Group II (n=20)</th>
<th>Group III (n=20)</th>
<th>Control (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leptin (ng/ml)</td>
<td>Mean ±SD 10.5±3.9</td>
<td>Mean ±SD 9.7±5.3</td>
<td>Mean ±SD 5.7±2.7</td>
<td>Mean ±SD 12.2±2.5</td>
</tr>
<tr>
<td>TNF-(\alpha) (pg/ml)</td>
<td>9.5±1.4</td>
<td>7.1±1.9</td>
<td>8.2±2.6</td>
<td>10.2±3.6</td>
</tr>
</tbody>
</table>

TNF-\(\alpha\): Tumor necrosis factor-\(\alpha\)

The difference of serum leptin levels between moderate COPD patients and severe COPD patients, in any group, was not statistically significant \([\text{Group I}: \text{moderate group} (8.0 \pm 5.3) \text{ng/ml}, \text{severe group} (14.7 \pm 5.9) \text{ng/ml}, P >0.05]; \text{Group II}: \text{moderate group} \ldots\)
(9.2±2.5) ng/ml, severe group (10.0±8.0) ng/ml, \( P >0.05 \); **Group III**: moderate group (1.5 ± 1.5) ng/ml, severe group (5.2± 3.5) ng/ml, \( P >0.05 \).

**Correlation analyses:**

*Correlation between leptin, TNF-α, and the nutritional parameters*:

Leptin levels were significantly correlated with BMI (\( r =0.524, P <0.05 \)), IBW% (\( r =0.448, P <0.05 \)), TSF (\( r =0.468, P <0.05 \)), MAC (\( r =0.507, P <0.05 \)), PA (\( r =0.367, P <0.05 \)), Alb (\( r =0.482, P <0.05 \)) and TF (\( r =0.312, P <0.05 \)) in COPD patients.

Leptin levels were significantly correlated with BMI and IBW% in both groups III and II: **Group III (COPD, with malnutrition, stable disease)** (BMI: \( r =0.560, P <0.05 \); IBW%: \( r =0.875, P <0.05 \)) and **Group II (COPD, without malnutrition, stable disease)** (BMI: \( r =0.597, P <0.05 \); IBW%: \( r =0.562, P <0.05 \)), but the correlation disappeared completely in **Group I (COPD, without malnutrition, acute state)** (BMI: \( r =0.152, P >0.05 \); IBW%: \( r =0.389, P >0.05 \)).

On the other hand, there was no significant correlation between nutritional parameters and TNF-α.

*Correlation between leptin, TNF-α, and the pulmonary function parameters*:

There was no significant correlation between leptin, and FEV1 (\( r =0.017, P >0.05 \)), FVC (\( r =0.021, P >0.05 \)), MIP (\( r =0.339, P >0.05 \)), MEP (\( r =0.252, P >0.05 \)) in COPD patients.

There was no significant correlation between TNF-α and FEV1 (\( r =0.141, P >0.05 \)), FVC (\( r =0.203, P >0.05 \)), MIP (\( r =0.282, P >0.05 \)), except in MEP which showed a significant positive correlation with TNF-α, (\( r =0.376, P <0.05 \)) in COPD patients.

**Discussion**

Chronic obstructive pulmonary disease, a worldwide cause of mortality and morbidity. Malnutrition is a common problem in many patients with chronic obstructive pulmonary disease (COPD), negatively affecting their exercise performance, health related quality of life and survival rates (*Wouters, 2002*).

Unexplained weight loss is common in patients with chronic obstructive pulmonary disease (COPD). COPD is a syndrome of chronic wasting, in part, associated with a chronic inflammatory response (*Wouters, 2002*).

Since leptin, an obesity gene product, is known to play important roles in the control of body weight and energy expenditure (*Bjorbaek & Kahn, 2004*), we investigated, in this study, serum leptin levels, along with circulating tumor necrosis factor-α (TNF-α) and
other nutritional parameters in chronic COPD patients and in healthy subjects; aiming to explore the role of serum leptin in the malnutrition of COPD patients, and to observe the changes of serum leptin levels during acute exacerbation of COPD, and also to investigate the relationship between leptin and TNF-α.

This study demonstrated that, serum leptin levels were significantly lower in weight-losing COPD patients than in controls and COPD patients without malnutrition. Similar results have been reported by Yuan et al., 2000 and Takabatake et al., 1999.

Leptin seems not to play an important role in weight loss in patients with COPD. The positive correlation between serum leptin levels and BMI in weight-losing COPD patients confirms that circulating leptin levels remain regulated even in patients in the cachexic status seen in COPD. Similar physiologic regulation of leptin is also noted in other malnutritional states such as anorexia nervosa (Grinspoon et al., 1996) and acquired immunodeficiency syndrome (Grunfeld et al., 1996, and Yang et al., 2006).

The mechanisms underlying the metabolic changes associated with the wasting process in COPD are complex. They involve interaction between cytokines and the hypothalamus and the direct effects of IL-1 and TNF alpha on peripheral tissues and liver (Tracey et al., 1988). TNF-α, a multifunctional cytokine, may play a potential role in the weight loss noted in COPD patients as well as in other cachectic patients with chronic wasting diseases (Takabatake et al., 2001 and Godoy et al., 1996).

Creutzberg and coworkers, reported that increased leptin concentrations related to the temporary disturbances in the energy balance during acute exacerbation of COPD (Creutzberg et al., 2000b). However, there was no statistically significant difference in serum leptin levels between COPD patients without weight loss during stable disease and acute exacerbation in our study. In their study, patients were treated with high dose glucocorticosteroids and the course of plasma leptin seemed to mimic the scheme of prednisolone administration.

Glucocorticosteroids in therapeutic dose have a stimulating effect on leptin concentrations via the induction of insulin resistance, as glucose and insulin are also able to induce leptin expression (Saladin et al., 1996).

Also, in our study, there was no statistically significant difference in serum leptin levels between COPD patients during stable disease and acute exacerbation, after the effect of high dose systemic glucocorticosteroid treatment was eliminated. Leptin levels were significantly correlated with BMI and IBW% in the patients with weight losing stable COPD, but the correlation disappeared completely in the exacerbation group.

These findings were in accordance with previous studies (Schols et al., 1999, and Yang et al., 2006). The dissociation of the normal feedback regulation of leptin by nutritional parameters might have been the results of the systemic inflammatory response during the exacerbation.
Also *Takabatake et al. 1999*, suggested that serum leptin levels were significantly lower in the COPD patients than in the healthy controls, which is in agreement with our results.

In addition, *Takabatake et al. 2001* studied the diurnal pattern of 24-hour leptin levels, and found that the diurnal pattern of 24-h leptin was present in both control subjects and non-cachexic COPD patients, but was absent in cachexic COPD patients. According to these data, it was suggested that the loss of circadian rhythm of circulating leptin has clinical importance in the pathophysiologic features in cachexic patients with COPD.

Several studies suggested that, cachexic patients with chronic obstructive pulmonary disease (COPD) show abnormalities of the autonomic nervous system (ANS), neuroendocrine function, and energy expenditure. Leptin has been implicated in the regulation of ANS, neuroendocrine function, and thermogenesis in humans, and therefore may play a role in mechanism of cachexia in COPD patients (*Argiles et al., 1997, and Mantzoros, 1999*).

It has been reported that TNF-α levels were higher in bronchial biopsies and induced sputum of COPD patients (*Kestings et al., 1996*). In addition, it has been shown that both circulating levels of TNF-α and TNF-α production by peripheral blood monocytes were increased in weight-losing stable COPD patients (*de Godoy et al., 1996, and Yang et al., 2006*).

In another study, no differences were seen in the concentrations of soluble TNF-α R55 and R75 between patients with exacerbated COPD and healthy subjects, and these concentrations did not change with exacerbation therapy (*Creutzberg et al., 2000b*).

In our study, there was no statistically significant difference in serum TNF-α levels between normal-weight stable COPD patients, weight-losing stable COPD patients, and patients with exacerbated COPD. But, there was significant difference in serum TNF-α levels between COPD patients and healthy controls, where its level was lower in COPD patients. There was no significant correlation between TNF-α and nutritional parameters in any group. These observations suggest that TNF-α seems not to play an important role in malnutrition and exacerbation in patients with COPD. These results are in agreement with *Figen et al., 2005*, who found that, there was no differences in TNF-α levels between COPD patients with low BMI(<20 kg/sq m) values and the remaining COPD patients, while a significant difference was found between COPD patients and healthy controls. A possible explanation of the absence of any difference in TNF-α values of COPD patients (group 1, 11 and 111) in our study could be that all groups of patients had similar levels of hypoxemia. These findings can also be interpreted to indicate that decreased levels of TNF-α are not associated with BMI changes and/or cachexia in patients with COPD, although the numbers of COPD patients in each subgroup were relatively small.

Since no direct correlations between circulating TNF-α levels and weight loss were found in patients with COPD, unidentified factors that could interfere with both the TNF-
α system and cachexia in COPD patients have been considered (Godoy et al., 1996). There are several speculations for the increased TNF-α levels in COPD patients. First, inflammation is the source of activation of TNF-α system (Tracey et al., 1988). Second, hypoxemia might contribute to the activation of the TNF-α system independently of airways inflammation (Takabatake et al., 1999). Third, the increased levels of circulating TNF-α could be related to the pathophysiology of right-sided heart failure associated with COPD (Takabatake et al., 1999). Our study had been demonstrated that serum levels of TNF-α were higher in weight losing COPD patients than in COPD patients of normal weight, and this is in agreement with (Considine et al., 1996 and DiFrancia et al., 1994). These studies have shown that impairment of lung function and hypoxemia tend to be more severe in cachectic patients with COPD than in noncachectic patients with COPD.

In our study, leptin levels were significantly correlated with nutritional parameters but not correlated with pulmonary functions in COPD patients. Several studies showed that TNF-α treatment increased leptin levels in rodents (Grunfeld et al., 1999), and human subjects (Zumbach et al., 1997, and Calikoglu et al., 2004).

Some studies did not find significant correlation between TNF-α and leptin (Takabatake et al., 1999, and Yuan et al., 2000). The discrepancy in these findings may be due to species differences or different stages of the disease. And this is in agreement with our results. Several studies conflicting results on the relationship between serum leptin and TNF-α levels. For example, Schools et al showed that a significant relationship existed between these parameters in patients with emphysema (Schols et al., 1999). In contrast, Takabatake et al. (1999), demonstrated that serum leptin levels did not correlate with serum TNF-α levels.

In our study, we did not find significant correlation between serum TNF-α and serum leptin in any group. Similar results have been reported by Takabatake et al., 1999, Yuan et al., 2000, and Yang et al., 2006. These findings suggested that leptin is not primarily under the control of the TNF-α system. Others demonstrated a relationship between inflammatory cytokines, such as TNF-α or IL-1, and leptin in inflammatory state. Leptin levels may be one mechanism by which anorexia is induced during acute inflammatory conditions (Sarraf et al., 1997). The normal leptin feedback mechanism can be disturbed by several factors. In animals, administration of endotoxin, TNF-α or IL-1, inflammatory cytokines known for their anorectic effects, resulted dose dependently in an increase in circulating leptin concentrations (Sarraf et al., 1997 and Grunfeld et al., 1996).

When compared to literature, it was proved that in patients with emphysema, in particular, a significant relationship between plasma concentrations of leptin and sTNF-R55 adjusted for fat mass and oral corticosteroid. Baseline plasma leptin concentrations were inversely related to baseline dietary intake and to the change in body weight after nutritional intervention. Temporary disturbances in energy balance related to increased leptin concentrations as well as to the systemic inflammatory response were also reported during acute exacerbations (Creutzberg et al., 2000b, and Wouters, 2002).
In this study, it was noticed that COPD patients, especially malnourished underweight persons (group III), had significantly lower BMI, lower ideal body weight and serum leptin (against expectations) levels than did the healthy control group, and this in agreement with Figen et al., 2005. Serum leptin concentrations were correlated with BMI, suggesting that low leptin levels in COPD patients may develop secondary to weight loss (Figen et al., 2005). In humans, there was variability in plasma leptin levels at each BMI group, suggesting that there are differences in its secretion rate independent of body fat. However, weight loss due to food restriction is associated with a decrease in plasma leptin in samples from obese humans who become insensitive to endogenous leptin production (Takabatake et al., 2000).

The advantage of analyzing plasma proteins in the assessment of nutritional status is that their concentrations are not influenced by body weight (Piitulainen et al., 2002). Malnutrition could be detected in COPD patients while they still have normal body weight. Albumin levels have been recommended as a means of detecting and monitoring protein calorie malnutrition, because levels vary directly with adequacy of intake (Veldee, 1994). Other than protein calorie malnutrition, the most common cause of low albumin levels is acute and chronic inflammation states (Figen et al., 2005).

In our study, as a biochemical marker of malnutrition, we analyzed plasma albumin, serum transferrin, and serum prealbumin, as well as triceps skin fold, mid-upper arm circumference and mid upper arm muscle circumference. Plasma albumin and other nutritional parameters were significantly lower in group III (COPD patients with malnutrition, stable disease) than in other groups. However, there were no differences in plasma albumin levels between COPD patients with a low BMI (<20 Kg/sq m) and the remaining COPD patients. These results are in agreement with Figen et al., 2005.

Available information suggests that muscle wasting is present in a large population of patients with COPD but its prevalence can be only be approximately as there are simple techniques to measure muscle mass (Debigare et al., 2001). The BMI was of limited value for determining changes in body composition and did not identify patients with skeletal muscle depletion, so, we did some anthropometric measures in addition to BMI e.g IBW, TSF, MAC and MAMC for more accurate assessment of the nutritional status of our cases (Veldee, 1994).

In summary, we evaluated serum levels of leptin and TNF-α in normal-weight stable COPD patients, weight-losing stable COPD patients, patients with exacerbated COPD and age-matched healthy controls. We found that circulating levels of plasma leptin were significantly lower in weight-losing stable COPD patients than in normal-weight stable COPD patients and circulating leptin levels correlated well with BMI in weight-losing stable COPD patients.

There was no statistically significant difference in serum leptin levels between COPD patients without weight loss during stable disease and acute exacerbation in our study. In addition, there was no significant correlation between TNF-α and leptin in any group.
We concluded that the physiologic regulation of leptin is maintained despite weight loss in patients with COPD, and that circulating levels of leptin are not controlled by the TNF-α system i.e. circulating leptin works independently of the TNF alpha system and does not primarily affect BMI in patients with COPD. However, decreased levels of circulating leptin may have some pathophysiologic roles in patients with COPD. And, a further longitudinal studies are indicated, on a larger scale, to confirm this cytokine(TNF alpha)-leptin hypothesis in malnutrition of COPD patients which may then open a novel approach to combat this significant comorbidity in COPD.

References


